JOURNAL OF AGRICULTURAL RESEARCH

VOL. 65 WASHINGTON, D. C., SEPTEMBER 1, 1942 No. 5

INFLUENCE OF CONTROLLED ENVIRONMENT AND NUTRITION ON THE RESISTANCE OF GARDEN PEA TO FUSARIUM WILT[1]

By WILBUR T. SCHROEDER, formerly *fellow*, and J. C. WALKER, *professor of plant pathology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

Resistance to fusarium wilt of pea (*Pisum sativum* L.), first noted by Linford (*7*),[2] was demonstrated by Wade (*19*) to behave as a single dominant Mendelian character. Although the disease has declined in importance with the development of resistant varieties, it remains of fundamental biologic interest in that it is in some respects an extreme case of the specialized parasitism common in the vascular fusarioses and differs noticeably in its temperature relations from other diseases in this group. Linford (*7*) and Snyder (*16*) showed that the growth of the pathogen, *fusarium oxysporum* f. *pisi* (Lindf.) race 1 S. & H. (*Fusarium orthoceras* App. and Wr. var. *pisi* Linford), on various media is similar to that of other vascular fusaria. Linford (*7*), however, found the optimum soil temperature for development of pea wilt to be distinctly below that for the growth of the fungus on potato-dextrose agar, while that in other vascular fusarioses is close to the optimum for linear growth of the parasite on agar substrate.

Walker (*20*) demonstrated that host resistance to cabbage yellows (*Fusarium oxysporum* Schlecht. f. *conglutinans* (Wr.) S. & H.), like that to pea wilt, is controlled by a single dominant Mendelian gene. Cabbage yellows, however, differs from the latter disease in that homozygous resistant individuals develop certain atypical symptoms at soil temperatures of 26° to 30° C. (*1*, *22*). At the same temperature range Linford (*7*) claimed that pea wilt was much reduced in severity. Anderson and Walker (*1*) found that penetration of the resistant cabbage occcurred through the epidermis and cortex of the root and hypocotyl, but that the organism seldom reached the stele. Walker and Snyder (*23*) observed that, in contrast to *F. oxysporum* f. *conglutinans*, the pea-wilt organism becomes established with difficulty in certain soil types.

The purpose of the present investigation was to study the effect of certain environal and nutritional factors on the development of pea wilt, particularly where the soil factors were eliminated. This was accomplished by growing a susceptible and a resistant variety of the host in artificially infested sand in which temperature and nutrition

[1] Received for publication January 14, 1942. This investigation is part of a study of the nature of disease resistance in plants, supported in part by the Wisconsin Alumni Research Foundation. Some assistance was provided from the personnel of Federal Work Projects Administration Project No. 65-1-53-2349.

[2] Italic numbers in parentheses refer to Literature Cited, p. 246.

could be regulated. Under these conditions a better understanding of the host-parasite relationship could be acquired which might in turn throw more light on the nature of disease resistance. All experiments were conducted in the greenhouse or laboratory at Madison, Wis.

MATERIALS AND METHODS

Two varieties of pea, Davis Perfection (wilt-susceptible) and Wisconsin Perfection (wilt-resistant), were used throughout. The isolate of *Fusarium oxysporum* f. *pisi* race 1 was obtained from W. C. Snyder of the University of California and all inocula used in this study were obtained from repeated substransfers of a single microconidial line derived from that culture. Five-pound glazed crocks, 5 inches high, were used. To each crock was added approximately 1,200 ml. of dry, fine, white silica sand, which previously had been washed in several changes of hot water followed by several rinsings in distilled water. Each crock was supplied with a siphon through which the sand was drained (fig. 1). The sand contained in each crock was wetted and sterilized for 6 to 8 hours at 15 to 18 pounds' pressure on 2 alternate days, and just prior to planting it was washed twice with distilled water by flooding each crock and draining through the siphon.

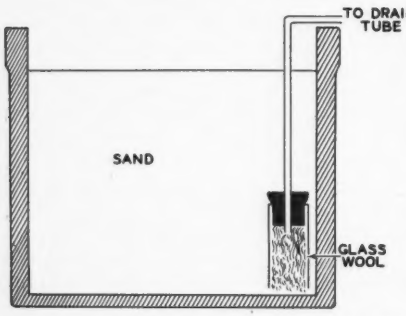


FIGURE 1.—Siphon drain for sand-culture crocks. The 2-inch piece of glass tubing filled with glass wool served to prevent the sand from clogging the drain.

Modifications of the nutrient solution formulated by Hoagland and Snyder (*4*) were used throughout. In the temperature experiments the basal solution was diluted to one-tenth concentration. The studies on the effect of nutrient concentration involved the dilute solution, the basal solution, and multiples of the latter. Hereafter, the diluted solution is designated as 0.1H, the basal as 1H, three times the basal as 3H, and five times the basal as 5H. No attempt was made to control the pH of the solutions. On alternate days approximately 500–600 ml. of nutrient solution was added to each crock and the excess solution drained off immediately.

The composition and molarity of the nutrient solutions at the basal concentration used in the sand-culture experiments were as follows:

Composition and molarity	*Milliliters to make 10 liters of nutrient*
$Ca(NO_3)_2$, 1M	50. 0
KNO_3, 1M	50. 0
$MgSO_4$, 1M	20. 0
KH_2PO_4, 1M	10. 0
NaCl, 1M	5. 5
A–Z [1]	2. 0

[1] The A–Z stock solution was composed of the following made up to 1,000 ml. with distilled water: H_3BO_3, 2,818 gm.; $CuCl_2.2H_2O$, 0.40 gm.; $ZnCl_2$, 0.030 gm.; $MnCl_2.4H_2O$, 0.390 gm.; and $FeCl_3.6H_2O$, 5,000 gm.

Inoculum was obtained by growing the fungus in 1-liter Erlenmeyer flask containing 200 ml. of Czapek's solution modified according to the following formula: KNO_3, 3gm.; KH_2PO_4, 1.0 gm.; $MgSO_4.7H_2O$, 0.5 gm.; KCl, 0.5 gm.; $FeSO_4$, trace; dextrose, 30 gm.; water, 1,000 ml. The inoculated flasks stood for 48 hours with frequent shakings each day, after which they were placed in a mechanical shaker for 3 to 4 days. After this period of agitation, the contents of each flask consisted of a very heavy suspension of microconidia and hyphal fragments. Inoculation was accomplished by adding 100 ml. of the original mycelial and spore suspension combined with 100 ml. of sterile 2-percent dextrose solution to each crock, draining the excess liquid, and thoroughly mixing the sand in each crock. Each control crock received 200 ml. of the sterile dextrose solution. The peas were then planted, usually eight seeds per crock, and flooded with the nutrient solution, which drained off immediately. The crocks were then placed in the respective temperature tanks. A second inoculation was made 3 to 4 days after emergence by adding 100 ml. of the mycelial and spore suspension to each crock, draining off the excess liquid, and flushing immediately with the nutrient solution.

In order to insulate the sand and thus maintain more complete temperature control, a circular ½-inch wire-mesh screen was suspended about ½ inch above the surface of the sand just after planting. A very thin layer of nonabsorbent cotton was placed over the screen. When the plants emerged through the mesh, the cotton was tucked around them. This method was more satisfactory than the use of granulated cork since the latter became wet when the nutrient solution was added to the sand and thus provided a suitable substrate for air-borne contaminants, particularly *Cephalothecium roseum* Cda.

SYMPTOMS AND DISEASE CRITERIA

The symptoms of pea wilt have been described in detail by Linford (*7*, *8*, *9*, *10*). The initial and most characteristic sign of this disease in susceptible plants is a downward curving of the stipules and leaflets, accompanied by a slight yellowing of the leaves and a superficial grayness suggesting an excessive development of waxy bloom. The lower internodes increase in diameter and the entire stem becomes rigid. After this stage the plant may wilt abruptly at the top, and the stem shrivel downward or, particularly under low soil-temperature conditions, it may turn yellow and wither slowly, leaf by leaf. Linford (*9*) has described, in plants grown aseptically in tubes of soil inoculated with a pure culture of the fungus, distortion and wilting of the leaflets followed by sudden collapse of the plant, sometimes accompanied by a water soaking of the collapsed parts and extensive cortical decay of the roots.

The progress of the disease was noted daily. Data were taken on the appearance of initial symptoms, complete wilting, and death. Complete wilting was designated as that stage in which all stipules and leaflets except those enclosing the terminal bud were either definitely wilted or dead. Indices were calculated according to the method used by Virgin and Walker (*17*, *18*) in which the index represents the average number of days from sowing to the particular stage of the disease concerned. The index for the initial appearance of symptoms was designated as the initial-disease index; that for

complete wilting, the wilt index; and that for death, the death index. On this basis, the more rapid the disease development became, the lower the index. Since neither complete wilting nor death occurred in all resistant plants, a disease-development index was used as the disease criterion. At a given period, plants were removed from the sand and divided into five arbitary classes designated as follows: 0, plants healthy; 1, no apparent stunting, but an incurving of stipules and leaflets, accompanied by off-color; 2, moderate stunting, incurving of stipules and leaflets and off color apparent to within three or four nodes of terminal bud; 3, severe stunting, only one to three uppermost nodes with green leaves remaining and possessing the characteristic incurving and off color (such plants approached the condition of complete wilting); 4, complete wilting and death. The disease-development index was calculated on the basis of the above classes by using the class figures as weighted values.[3]

ENVIRONAL STUDIES

Linford (*7*) indicated that the most rapid and severe development of pea wilt occurred at a soil temperature of 21° to 22° C. He based his conclusions on the percentage of diseased plants at the various soil temperatures on successive-day intervals following planting. Using a wilt index, Virgin and Walker (*17*) compared the soil-temperature relations of the near wilt disease of pea (*Fusarium oxysporum* Schlecht. f. *pisi* (Sny.) race 2 S. and H.) with those of pea wilt and corroborated Linford's observations on wilt, although the differences between 20°, 24°, and 30° were not very large.

Early experiments involving a study of methods for obtaining uniform infection of peas in sand artificially infested with the pea wilt organism indicated that the optimum temperature for disease development was not convergent with that in soil. Furthermore, it was observed that at temperatures of 24° C. and higher, resistant plants were stunted, and they developed symptoms characteristic of the initial stages of the disease described by Linford (*7*) for susceptible plants. Two experiments were therefore set up to determine the influence of sand temperature on development of the disease in susceptible and resistant varieties. The first experiment was started in the greenhouse on December 2 and final notes were taken on January 17; the second experiment was started on January 31 and concluded on March 18. The chief variable in the two experiments, therefore, was light, the length of day being shorter and the intensity of light usually lower in the first experiment. In experiment 1 a parallel series was run in virgin soil in which inoculum increased on sterilized barley kernels was incorporated at approximately the same rate as that used by Snyder (*16*). The soil was maintained at approximately 60 percent of its water-holding capacity by the addition of distilled water on alternate days. Uninoculated soil served as a control. Three inoculated and two control crocks of each variety in each substrate were planted. Crocks and tanks were arranged

[3] To obtain the index the number of plants in each class was multiplied by the class number and the sum of the products of each class was then multiplied by 100. The product was then divided by 4 times the total number of plants in each treatment to obtain the disease-development index. Thus, when all the plants were healthy, the index was 0, when all the plants were completely wilted the index was 100, while intermediate stages of the disease were represented by intermediate index figures. It is important to note that the disease-development index figure increases in value with increase in disease severity while in the initial-disease index, wilt index, and death index the reverse relation holds

at random to compensate for any variation in quantity and intensity of light in the greenhouse. Tank temperatures were maintained at 15°, 18°, 21°, 24°, 27°, and 30°, and the cotton insulation in each crock permitted these temperatures to be held to within ½° in the sand and soil. The air temperature was held between 22° and 24°, except for about 4 hours during midday on sunny days, when it went as high as 28°.

INFLUENCE OF TEMPERATURE ON THE DISEASE IN SUSCEPTIBLE PLANTS

The influence of sand temperature on the development of wilt was markedly different from that observed by Linford (*7*) for soil. From the data given in table 1 it is to be seen that the time required for complete wilting to occur was significantly less as the temperature rose from 15° to 30° C. The difference between the wilt indices at 27° and 30° was not significant in experiment 1 and just barely so in experiment 2. The time required for complete wilting in the latter was the longer at each temperature. Thus the optimum temperatures for complete wilting in these trials were 27° and 30°.

TABLE 1.—*Wilt indices of Davis Perfection (susceptible) peas in two different experiments at various sand temperatures*

Experiment No.	Duration of experiment	Wilt index (days) at sand temperatures of—						Minimum significant difference (19:1)[1]
		15° C.	18° C.	21° C.	24° C.	27° C.	30° C.	
1	Dec. 2 to Jan. 12	31.3	27.9	24.2	20.6	16.8	16.5	2.10
2	Jan. 31 to Mar. 18	41.1	33.6	29.8	25.6	19.4	18.3	1.03

[1] Minimum difference required for significance between the indices at various temperatures in a given experiment.

The progress of disease development (fig. 2) showed the same trend in the three indices, although the intervals between the three stages of wilt development decreased slightly with the rise in temperature. While any one of the three stages could be used as a disease criterion, complete wilting appeared to be the one most accurately determined. A comparison of the initial-disease indices in sand and soil presents an interesting picture (fig. 3). As previously indicated, the differences among the indices at the various sand temperatures were all significant except at 27° and 30° C. In the soil, however, the appearance of initial symptoms at 18°

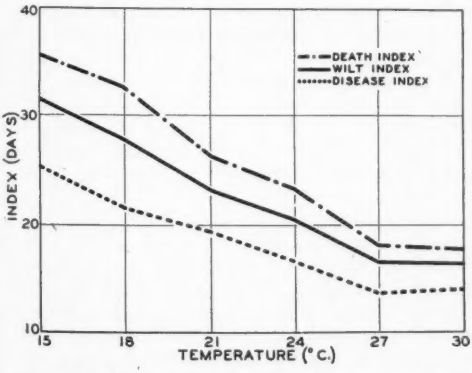


FIGURE 2.—Comparison of the initial-disease index, the wilt index, and the death index in Davis Perfection (susceptible) peas at various temperatures in artificially inoculated sand.

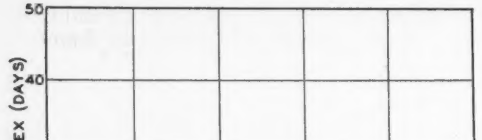


FIGURE 3.—The influence of temperature on the initial-disease index in Davis Perfection (susceptible) peas grown in artificially inoculated sand and soil at various temperatures.

and 21° was significantly earlier than at any of the other temperatures. At 15° the symptoms were decidedly retarded, while at 24°, 27°, and 30° they were intermediate in rate of development between those at 15° and at 18° and 21°. Thus at 15°, 18°, and 21° the disease developed at approximately the same rate in both sand and soil, but at 24°, 27°, and 30° a divergence occurred with the result that the optimum temperature for disease development in sand was much higher than that in soil.

INFLUENCE OF TEMPERATURE ON THE DISEASE IN RESISTANT PLANTS

Several attempts have been made to induce the development of wilt in resistant varieties. Linford (*9*) failed to break down inherent resistance by pruning and otherwise injuring the roots of plants already established in wilt-infested soil. Decortication of the roots of transplants before placing in infested soil, as performed by Linford (*9*) and Wade (*19*), resulted in no manifestation of the disease other than a dropping of a few of the lower leaflets, although both workers state that the fungus was found sparingly in the cortex but not in the stele of the root. A similar situation was observed by Walker (*21*) in the resistant Alaska variety grown in soil and on soil-extract agar. Snyder (*16*) observed leaf symptoms on the resistant Bruce variety suggestive of wilt, but failed to recover the fungus from such plants. By applying a barley-grain culture of the fungus to wounds in the aerial parts of resistant and susceptible varieties, Linford (*10*) obtained symptoms which simulated those typical of the disease in 6.7 and 13.3 percent of the plants, respectively. The fungus, however, extended only a few millimeters vertically from the wound. In the two experiments previously described Wisconsin Perfection (resistant) peas were grown in a parallel series. Disease development in this variety will be considered next.

The symptoms in resistant plants were found to differ from those in susceptible plants in degree rather than in kind. The initial symptoms were quite similar in that the normal color of the lower leaflets and stipules gave way to a slight yellowing, followed by withering and death. As a given leaflet withered younger stipules and leaflets assumed a superficial grayness and became incurved about three nodes in advance of that at which leaves were dying. The dead leaves abscised at about the same rate as those of susceptible plants grown at 15° and 18° C. but more readily than those of susceptible plants grown under temperatures conducive to rapid wilting. Under ideal conditions, these symptoms gradually proceeded to the

top of the plant until all fully expanded leaves had withered and died. As in susceptible plants, the lower internodes increased in diameter and the entire shoot became more rigid, while stunting and shortening of the internodes were also quite characteristic. Under less favorable conditions, such as lower temperatures, the characteristic off color and incurving of the stipules and leaflets developed occasionally, but they were confined to the lower half of the stem and occurred without any apparent stunting of the plant. A certain amount of cortical decay existed in the roots of such plants.

The influence of temperature on disease development in Wisconsin Perfection was not as marked nor as uniform as in susceptible Davis Perfection. The initial-disease indices are given in table 2 while the percentages of completely wilted plants at various intervals for each temperature are represented in figure 4. The temperatures of 24°, 27°, and 30° C. were uniformly effective in bringing about early appearance of initial symptoms, while 27° and 30° appeared to be slightly more favorable than 24° for the early development of the complete-wilt stage. The symptoms appeared later at 21° and were greatly retarded at 15° and 18°.

TABLE 2.—*The influence of temperature on the appearance of initial symptoms in Davis Perfection (susceptible) and Wisconsin Perfection (resistant) peas grown in artificially inoculated sand*

Experiment No.	Duration of experiment	Variety	Initial-disease index[1] (days) at sand temperatures of—					
			15° C.	18° C.	21° C.	24° C.	27° C.	30° C.
1	Dec. 2 to Jan. 12	Davis Perfection	25.3	21.8	19.4	16.8	13.7	14.0
		Wisconsin Perfection	31.9	30.2	23.4	14.9	14.4	14.8
2	Jan. 31 to Mar. 18	Davis Perfection	28.2	21.9	18.2	16.2	14.5	14.4
		Wisconsin Perfection	33.5	30.5	26.8	20.3	19.3	20.9

[1] Based on the average of 3 replicates.

At the conclusion of experiment 1, those plants that had not wilted completely were removed, washed free of sand, surface-sterilized for 5 to 8 minutes in a 0.9-percent solution of sodium hypochlorite, and nodal platings were made on acidified potato-dextrose agar. A fungus resembling *F. oxysporum* f. *pisi* race 1 with respect to cultural characters was isolated from every plant, although the nodal location of the fungus was irregular. The majority of isolations were from the cotyledonary and second nodes. In some plants the fungus was recovered from as high as the fifth node, but was rarely recovered from that section of the taproot located about 1 cm. below the cotyledonary node.

Forty-five days after planting, those plants of the resistant variety that had not wilted completely in experiment 2 were removed from the sand and the disease-development index was determined prior to planting the nodes on acidified potato-dextrose agar. The indices were as follows:

Temperature (°C.):	*Disease-development index*
15	26.3
18	30.7
21	47.4
24	73.7
27	91.3
30	92.1

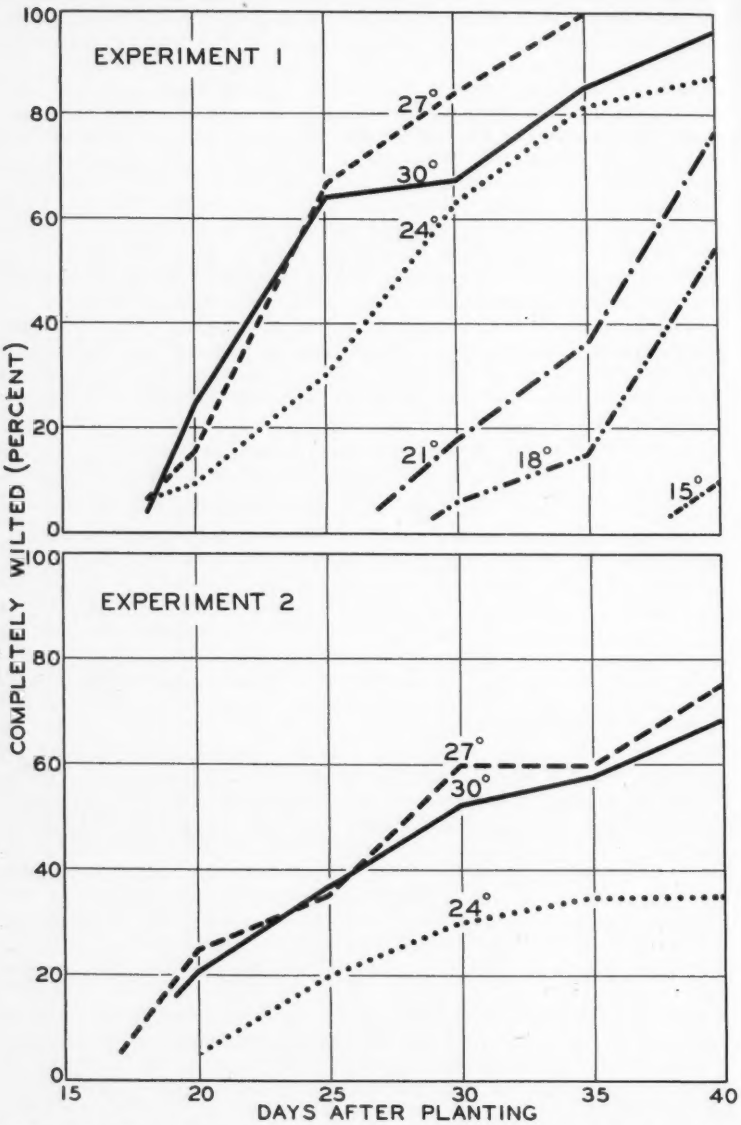


FIGURE 4.—The influence of sand temperature on the percentage of plants reaching the complete-wilt stage in Wisconsin Perfection (resistant) peas grown in artificially inoculated sand.

The most severe development of the disease occurred at 27° and 30° C., with a decrease at 24° and a still further decrease at 21°, while at 15° and 18° the majority of the plants showed merely the characteristic incurving and off color at the lower nodes. The results of the nodal isolations conformed closely to the disease-development indices. Those plants classified as healthy did not yield the organism. In diseased plants at 15°, 18°, and 21° the pathogen had not progressed farther than the second node and was sparse in the root. At 24°, 27°, and 30° the organism was recovered from the roots and second node of all plants and from the fourth, fifth, or sixth nodes of some.

INFLUENCE OF AIR TEMPERATURE ON DISEASE DEVELOPMENT

In view of the marked differences effected by various sand temperatures on the development of pea wilt, a study was made of the influence of different air temperatures on the progress of the disease in the susceptible and resistant varieties grown at two constant sand temperatures. Triplicate plantings of each variety were made at sand temperatures of 20° and 28° C. in each of the following air temperatures: 16°, 20°, 24°, and 28°. Control crocks were planted in duplicate. Cotton insulation was used in all crocks. The results in table 3 show no significant difference in wilt indices at the four air temperatures when the sand temperature was 28°. When the sand was at 20° complete wilting was definitely retarded at 16° air, as compared with air temperatures of 20°, 24°, and 28°. Of the last three air temperatures wilt was significantly the more rapid at 24°. However, at air temperatures of 20°, 24°, and 28° the modification of disease expression occasioned by shifting the sand temperature from 20° to 28° or vice versa was greater than that between 20° and 28° air temperature at either soil temperature. The fact that the rate of wilt development in the sand held at 28° was not influenced by air temperature is explained in part by the observation cited elsewhere in this paper that complete wilting in susceptible plants in sand at 27° and 30° was the result of a different set of host-parasite relations than that in plants grown in sand at 24° and lower temperatures.

TABLE 3.—*Development of the complete-wilt stage in Davis Perfection (susceptible) peas grown in artificially infested sand at two sand temperatures in each of four air temperatures*

Sand temperature (° C.)	Wilt index at air temperature of—				Minimum significant difference (19:1)
	16° C.	20° C.	24° C.	28° C.	
20	[1] 30.7	27.7	25.9	26.7	1.6
28	17.7	18.0	17.8	20.5	[2]

[1] Average of 3 replicates.
[2] Calculated *F* value indicated no significant differences among the four air temperatures.

TEMPERATURE IN RELATION TO THE GROWTH OF THE FUNGUS

The optimum temperature for growth of *F. oxysporum* f. *pisi* race 1 as measured by radial expansion on agar media of diverse composition has been shown by Linford (*7*) and Snyder (*16*) to be around 28° C. Because of the inoculation technique employed in these experiments, it was deemed advisable to study the relation of temperature to growth

of the fungus in a nutrient solution having the same salt concentration and balance as that used for the growing plants. The 0.1H nutrient solution was made up with 2 percent dextrose, and 50 ml. of solution were added to each 250-ml. Erlenmeyer flask. Six inoculated flasks were placed at each of nine constant temperatures. At the end of 14 days, three flasks at each temperature were removed and the mycelial contents of each flask weighed; the other set of three was weighed at the end of 23 days. Prior to weighing, the mycelial growth was filtered, washed four times with distilled water, and the mats dried at 74° C. The pH of the filtrates at each temperature was not appreciably different from the original pH 4.35 in the sterile solution.

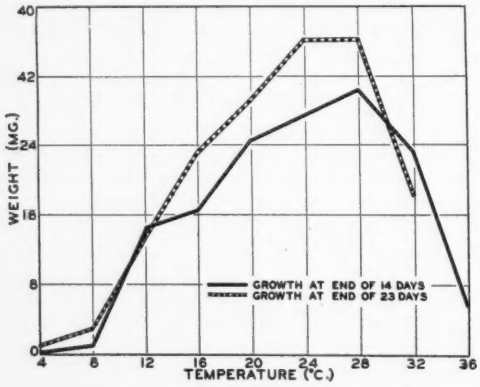


FIGURE 5.—Average weight of the mycelium of *F. oxysporum* f. *pisi* race 1 grown in 0.1H nutrient solution supplemented with 2 percent dextrose, at nine constant temperatures.

The results are shown graphically in figure 5. At the end of 14 days, 28° appeared to be the optimum temperature, whereas, at the end of 23 days, 24° and 28° produced an equal amount of growth. The small amount of growth produced might have been due to the very low concentration of nutrient salts. The thermal relation of the fungus to this medium was thus quite similar to that reported for potato-dextrose agar.

INFLUENCE OF SAND TEMPERATURE ON THE HOST-PARASITE RELATIONSHIP

It has been assumed that the influence of temperature on the development of fusarial wilts is primarily due to its direct effect upon the pathogen. Pea wilt has been a notable exception when plants were grown on infested soil, but the results just presented show that when soil is eliminated and infested quartz sand used the thermal relations do not differ from those of other vascular fusarioses. It cannot be definitely ascertained from the experiments reported herein whether or not the increased severity in disease development at 27° to 30° C. is due to the influence of these higher temperatures on the fungus, on the host, or on both. Nevertheless, certain observations on the development of the disease at the different sand temperatures do provide a better understanding of the relationship between host and parasite. Each plant in experiment 2 that had reached the complete-wilt stage was removed along with a corresponding control plant for further study. The smallest and the largest diameters of the first internode of each plant were measured. Root and nodal platings were then made with each diseased plant. The first internode of the completely wilted Davis Perfection (susceptible) plants was significantly larger at each temperature than that of corresponding healthy

control plants (table 4). A comparison of the differences in diameter at the various temperatures showed this to be greatest at 21°,

TABLE 4.—*Comparison of the diameters of the first internodes of wilted and healthy Davis Perfection (susceptible) plants at six different temperatures*

Condition of plant	Average diameter (mm.) [1] at temperatures of—					
	15° C.	18° C.	21° C.	24° C.	27° C.	30° C.
Completely wilted	3.09	3.16	3.46	3.45	3.51	3.47
Healthy control	2.90	2.78	2.75	2.91	3.06	3.11
Difference	.19	.38	.71	.54	.45	.36

[1] Minimum significant difference in comparing (19:1) temperatures within the healthy or inoculated group or between healthy and wilted plants within any temperature is 0.18 mm.

which is near the optimum for the growth of the host (7). The wilted plants of the resistant variety showed an increase in diameter of the first internode which was of the same order as that found in the susceptible variety. When wilted resistant or susceptible plants were measured it was observed that the first and second internodes were fully rounded, in contrast to those of healthy plants which exhibited definite furrowing due to schizogenous lacunae in the cortical parenchyma. Microscopic examination of transections of healthy and wilted plants at corresponding locations in the first internode revealed that the lacunae had not developed or, at least, were greatly retarded in the latter group. It would appear, therefore, that this host reaction to fungus invasion is common to resistant and susceptible forms and its relation to temperature is a function of the influence of the latter on the host rather than on the pathogen.

The progress of the fungus through the axis of the plant was measured by root and nodal isolations as each particular plant reached complete wilting since this stage represented the ultimate expression of the interaction of host and parasite. The results (table 5) show

TABLE 5.—*The influence of temperature on the advance of the pea-wilt organism in the stems of Davis Perfection (susceptible) plants at the complete-wilt stage*

Sand temperature (°C.)	Total plants	Plants in which the fungus had reached the node indicated by numbering from the tip downward—										Average of nodes in the plant at the complete wilting stage
		Tip	1	2	3	4	5	6	7	8	9	
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Number*
15	19	0	0	10.5	0	5.3	10.5	10.5	31.6	21.1	10.5	10.3
18	21	0	23.8	38.1	14.3	4.8	19.0	4.8	.....	.....	.....	8.6
21	22	31.8	31.8	27.3	9.1	.....	.....	.....	.....	.....	.....	8.1
24	21	61.9	19.0	19.0	.....	.....	.....	.....	.....	.....	.....	7.9
27	21	15.0	15.0	30.0	20.0	15.0	5.0	.....	.....	.....	.....	6.7
30	13	7.7	15.4	30.8	30.8	15.4	.....	.....	.....	.....	.....	6.5

that at 24° the fungus progressed to the tip in a greater percentage of plants than at any other temperature, while the closest approach to this situation occurred at 21°. The progress of the organism was definitely slower at 27° and 30° and still more so at 15° and 18°.

The average number of nodes per plant at the time of complete wilting was inversely proportional to the temperature. It is significant, however, that the organism reached the terminal node in fewer plants at 27° and 30° than it did at 24° and 21°, in spite of the fact that it had fewer nodes through which to travel and the additional fact that plants wilted at 27° and 30° were usually somewhat shorter than those that wilted at 24°. This would indicate that, insofar as this phase of host-parasite relationship is concerned, the influence of temperature on the pathogen is not strictly parallel to its effect on fungus growth.

The development of the disease beyond the appearance of initial symptoms was quite variable at the different temperatures, although the ultimate result was a withering and drying of the fully expanded leaves, followed by the death of the terminal bud. Once the initial symptoms appeared, wilting was most rapid at 27° and 30°, the uppermost leaves withered without any apparent change in color, and leaf abscission seldom occurred. At 21° the leaves of affected plants wilted more slowly and definite color changes preceded the death of each stipule and leaflet, while abscission of dead leaves occurred in most instances. Affected susceptible plants at 21° and at lower temperatures were very similar in appearance to resistant plants at 27° to 30° (fig. 6). Complete wilting in resistant plants seldom occurred at 15°, and when it did so it was characterized by a very slow necrosis and abscission of the leaflets until all but the terminal bud were dead.

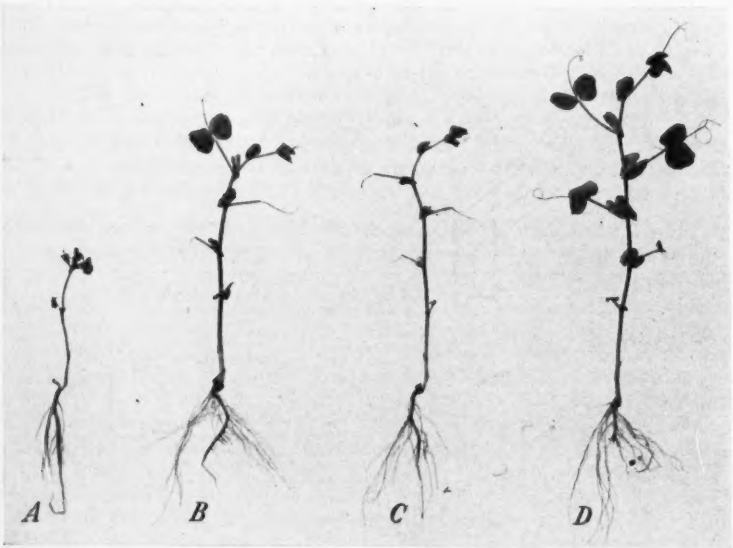


FIGURE 6.—A comparison of diseased pea plants of a susceptible and a resistant variety 23 days after planting, when grown at two different sand temperatures: *A* and *B*, Davis Perfection (susceptible) grown at 27° and 21°, respectively; *C* and *D*, Wisconsin Perfection (resistant) grown at 27° and 21°, respectively. All corresponding control plants approximated in size the plant on the extreme right.

An important characteristic of the disease in sand culture was that at 27° and 30°, which were the optima for the development of complete wilting, cortical necrosis of the roots was usually severe. Necrosis was progressively less prevalent as the temperature decreased. This is a phase of the disease which has seldom been mentioned by other workers who have used soil culture but is suggestive of a case described by Linford (*9*) on plants grown aseptically on inoculated soil. In view of the fact that the most rapid vascular invasion occurs at 24° and 21°, it may be that vascular invasion is responsible for the first appearance and most rapid advance of the disease in soil at 21°, while in sand where higher temperatures promote cortical invasion the higher optimum for complete wilting may be due in part to the injection of the cortical phase into the disease picture. In any case it is an interesting example of a shift by change in thermal environment from a more strictly vascular disease to one which is both vascular and cortical. It was not uncommon to find roots with extensive cortical decay in which healthy white lateral roots emerged through the necrotic area and served to delay the wilting of the above-ground stem and leaves. Such plants were found in the suceptible variety most commonly at 15° and in the resistant variety at 27° and 30° (fig. 7).

NUTRITIONAL STUDIES

RELATION OF INCREASED NUTRIENT CONCENTRATION TO DISEASE DEVELOPMENT

The experiments on the relation of sand temperature to pea wilt established a higher optimum for disease development than that previously recorded for infested soil and indicated that resistance to the disease could be partially broken down, especially at 27° and 30°. Concomitant with the increased disease development at these temperatures was a pronounced cortical decay of the roots of both resistant and susceptible plants heretofore unassociated with pea wilt, except in one instance cited by Linford (*9*). Smith and Walker (*14*) showed that multiple concentrations of the nutrient solution described by Hoagland and Snyder (*4*) have a retarding effect on infection and severity of aphanomyces root rot of pea (*Aphanomyces euteiches* Drechs.) in which severe cortical decay of the roots and lower stem occurs. They obtained no infection at nutrient concentrations four and five times that used by Hoagland and Snyder (*4*).

In a preliminary experiment conducted during October 1940, at a sand temperature of 27° with two concentrations of the nutrient solution, 0.1H and 1H, it was observed that the appearance of complete wilting of the susceptible plants was delayed at the higher nutrient concentration. Likewise, the disease was regarded in its development and was much less severe in the resistant plants supplied with the higher concentration (fig. 8). Since observations indicated that cortical decay was quite characteristic of pea wilt in both susceptible and resistant varieties grown in sand at 27° and 30° C. and supplied with a dilute nutrient solution, the effect of higher nutrient con entrations on the development of pea wilt was studied. With but few exceptions, the method used was essentially the same as that in the sand-temperature work. The sand was held close to 21° and 27°, respectively, since these two temperatures effected marked differences

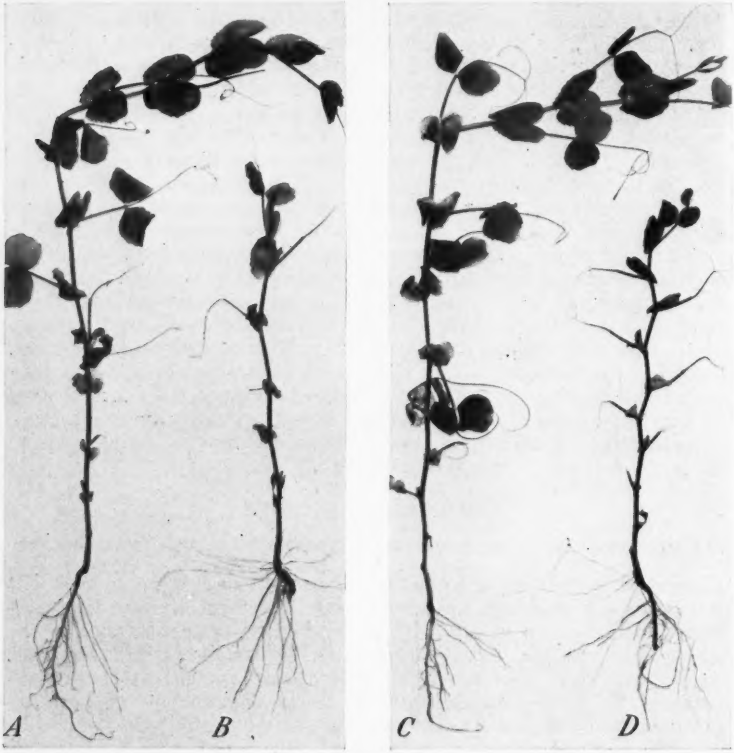


FIGURE 7.—Regeneration of the root system in peas following pronounced cortical decay produced by the wilt organism; this was found most commonly in wilt-susceptible peas grown at low sand temperatures and in wilt-resistant peas grown at high sand temperatures: *A*, Davis Perfection (susceptible) plant grown in uninoculated sand at 15° C.; *B*, Davis Perfection plant grown for 40 days in inoculated sand at 15°; *C*, Wisconsin Perfection (resistant) plant grown in uninoculated sand at 27°; *D*, Wisconsin Perfection plant grown for 40 days in inoculated sand at 27°.

in the development of the disease in both the resistant and the susceptible varieties. Two experiments were conducted, one from January 18 to March 5, the other from March 12 to May 4. The air temperature was maintained at 22° to 24° except on sunny days when it sometimes rose to 28° or slightly above for 4 to 6 hours. All inoculated crocks were run in triplicate, and the control crocks in duplicate. The progress of the disease was measured by the wilt index, except in the resistant variety in the second experiment, where the disease-development index was used because not enough complete wilting occurred to show the real effects among the nutrient concentrations.

Air-dry weights of the roots and stems of the healthy plants grown in the various concentrations of nutrient solution gave no indications of significant differences, except that, in general, the weights of the

stems of roots of plants growing at 21° were greater than those of the plants growing at 27°. Seedling emergence at 27° was 2 or 3 days earlier than at 21°. Plants growing in the 5H nutrient solution had smaller leaves, were somewhat shorter, and were distinctly darker green in color. No difference was observed in the size of the plants grown in the other nutrient concentrations, but the plants in the 0.1H solution possessed a lighter green color than those in the 1H, 3H, or 5H solutions.

It may be well to consider the effect of nutrients on disease development first at 21°, which is near the optimum for host development. The reaction at this temperature as a whole may then be compared

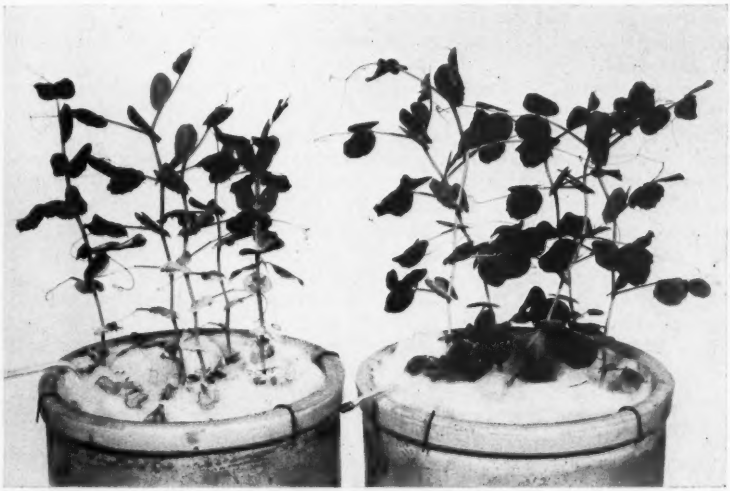


FIGURE 8.—Effect of nutrient concentration on the development of symptoms in Wisconsin Perfection (resistant) peas grown for 23 days at a sand temperature of 27° C: *A*, supplied with 0.1H solution; *B*, supplied with 1H solution. Note the leaf abscission and erect habit of stems in *A*.

with that at 27°, which is near the optimum for the pathogen and above the average of the range ordinarily encountered for any protracted period by peas in nature. The wilt indices of the susceptible plants are given in table 6. At 21° there was no significant difference

TABLE 6.—*The effect of nutrient concentration on the development of wilt in Davis Perfection (susceptible) peas grown in artificially infested sand*

Sand temperature (°C.)	Period of test	Wilt index at nutrient concentration—				Minimum significant difference (19:1)
		0.1H	1H	3H	5H	
21	Jan. 15–Mar. 5	25.6	26.6	22.3	23.8	[1]
21	Mar. 12–May 4	26.2	28.2	36.0	[2] 40.8	
27	Jan. 15–Mar. 5	18.6	18.6	15.1	13.3	3.65
27	Mar. 12–May 4	21.1	24.9	18.6	11.4	3.75

[1] Calculated *F* value indicated no significant differences among the 4 nutrient concentrations.
[2] 16.6 percent of the plants were diseased but not completely wilted at the end of the experiment.

between wilt indices at the four nutrient levels in the midwinter, short-day, low-light-intensity run. However, in the longer-day run with better light there was a definite increase in the wilt index and thus a slower development of disease with increase in nutrient concentration. At this temperature there were so few of the resistant plants which reached the complete-wilt stage that it was impossible to calculate comparable wilt indices and data are therefore not presented in the table. In both of the tests with resistant plants at 21°, however, the disease was most severe at 0.1H, less serious at 1H, and absent at 3H and 5H. At 21°, therefore, the increase in nutrient concentration had a depressive effect on disease except in the susceptible strain in midwinter when light was poor and days were short. Nutrition thus had the same general influence on wilt at this temperature as described for root rot (*Aphanomyces euteiches*) by Smith and Walker (*14*).

The picture at 27° was quite different. Severe cortical necrosis was evident on roots and lower stem internodes of susceptible and resistant plants at the two highest concentrations, 3H and 5H (fig. 9). There was a definite decrease in the wilt index and thus an increase in rate of wilt appearance with increase in nutrient level in susceptible plants (table 6) in both light periods at this temperature. This held true also for the short-day, low-light run with the resistant variety. It will be noted, however, that in the spring run with the susceptible variety (table 8) the disease developed significantly more slowly at 1H than at either 0.1H or 3H. This was even more pronounced in the resistant variety where the cortical decay was greatest at 3H and 5H. The result with the 0.1H and 1H concentrations coincided with that reported for the preliminary trial in October and illustrated for the resistant variety in figure 8. The plants at the 3H and 5H levels, however, showed increasingly severe necrosis and more rapid wilting. The resistant plants in the spring run at 21° and at 27° were removed at the end of the experiment and disease-development indices determined (fig. 10). The widely different reaction to nutrient concentration at the two temperatures is quite evident. At 21°, where vascular invasion was most prominent, there was a progressive decline in disease with increase in nutrient concentration. At 27°, on the other hand, where cortical decay entered the disease complex the rate of disease development declined when the nutrient level was raised up to 1H but increased sharply as the concentration rose above that level.

It is thus quite clear that temperature and nutrition each have an influence upon the type of disease reaction which prevails in sand culture. Generally speaking, under conditions of light and temperature favorable to the host (21°) a truer vascular disease prevails and increase in the concentration of the nutrient retards the pathologic development in the susceptible and particularly in the resistant variety. At the optimum temperature for the pathogen (27°) cortical decay of the root system is the predominant disease reaction while vascular invasion is secondary. Moreover, the combined effect is most severe in very weak (0.1H) and in very concentrated nutrient levels (3H and 5H) and least severe at an intermediate level (1H). This pronounced appearance of cortical invasion in a fusarial disease

ordinarily regarded as quite strictly vascular by a shift in environment tends to break down any definite line between cortical and vascular fusaria. It recalls, furthermore, the situation in certain other fusarial

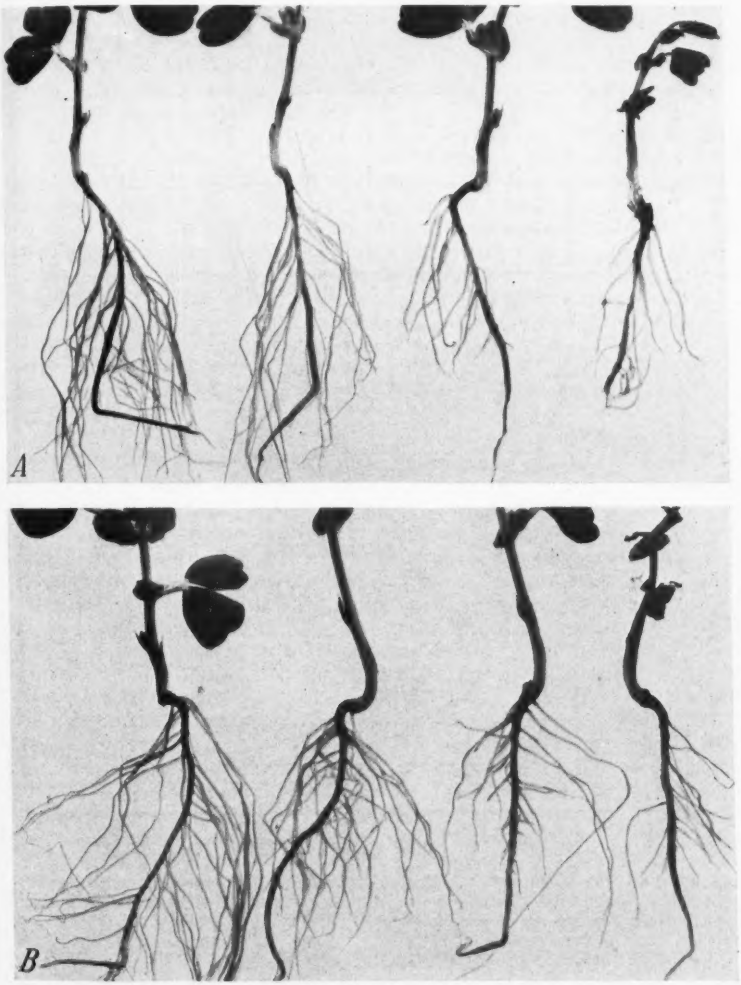


FIGURE 9.—The effect of nutrient concentration on the development of cortical decay in sand held at 27° C. *A*, Davis Perfection (susceptible); *B*, Wisconsin Perfection (resistant). From left to right, plants grown in nutrient concentrations of 0.1H, 1H, 3H, and 5H, respectively.

wilts, such as that of watermelon, in which at certain stages in host development the disease is predominantly cortical and necrotic while at another stage it becomes chiefly vascular and hypoplastic (*3*, *24*).

478749—42——2

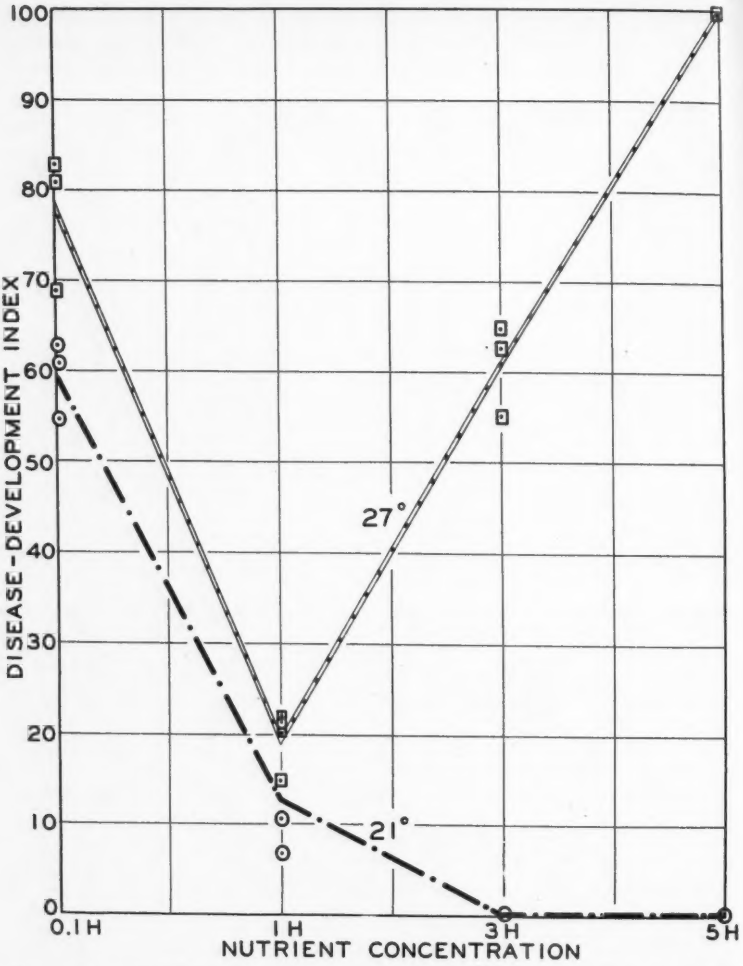


FIGURE 10.—The effect of four different nutrient concentrations on wilt development as shown by the disease-development index in Wisconsin Perfection (resistant) peas grown in sand held at 21° and 27° C.

PATHOLOGICAL ANATOMY

Previous histological examinations of the root systems of resistant plants grown under ideal conditions for soil infection failed to reveal the presence of the parasite in the stele (*7*, *21*). Walker (*21*) found invasion in the outer cortical cells of resistant Alaska seedlings to be meager in contrast to that in susceptible roots when grown on soil-extract agar previously inoculated with the parasite. Thus, it was suggested that resistance to pea wilt in plants grown in soil or on

artificial media was expressed in the root-tip region. In this study, however, extensive cortical decay in both resistant and susceptible plants was observed. Nodal isolations of resistant plants affected with the disease indicated that the organism had progressed to as high as the sixth node. In view of these facts, histological examination of the affected resistant plants was undertaken to determine the location and extent of invasion by the parasite, and to observe any notable reaction between host and parasite that might differentiate the resistant from the susceptible plants.

Plants were removed from the sand, usually at the complete-wilt stage, and the desired sections were placed in either formol-acetic-alcohol or dioxan fixative. The staining procedure followed throughout was a combination of Mayer's haem-alum and orange G in clove oil (*5, p. 78*). Sections were cut 10 to 15 microns in thickness. The presence of the organism in the tissue was verified by plating out a part of the surface-sterilized tissue above and below the section placed in the fixative. Pathogenicity tests of some of these isolates covering a varied range of cultural characteristics indicated the same difference in symptom expression between resistant and susceptible plants as when the original culture was used; no marked difference in virulence between the isolates of resistant plants and the original culture was observed.

Penetration of the roots of both varieties was not limited to the root-tip region. In fact, many infected roots showed a sound meristematic region in contrast to that part behind the tip. Occasionally roots were found in which the upper extremity showed pronounced cortical decay, and the region between it and the growing point exhibited a complete collapse of tissue not accompanied by any water soaking. In many instances, especially at the higher temperatures, the taproot and many laterals were decidedly browned and water-soaked. Transections of such roots showed pronounced cortical and stelar penetration (pl. 1). Inasmuch as the fungus was found in the phloem fibers it apparently encountered little difficulty in passing through the endodermal and pericyclic region of these roots (pl. 4, *A*). Sections of resistant roots were frequently found in which the parasite occurred in the outer rows of cortical cells (pl. 1, *A* and *B*). In such cases stelar penetration probably occurred at a lower level where cortical decay advanced to the stele. Hyphae were found in the xylem vessels of resistant plants as high as the first and second internodes (pl. 3, *A*). Nodal isolations indicated it had progressed still further in some plants.

No differences were found between varieties with respect to the enlargement of the lower internodes (pl. 2, *A* to *D*). This might be accounted for by an increase in cambial activity, especially in the plane of the polar bundles, which resulted in an increase in phloem and xylem tissue. Evidence of proliferation of xylem vessels and parenchyma in the region of the phloem fibers was also observed (pl. 4, *B*). These observations agree somewhat with those of Linford (*10*), wherein he noted a stimulation of xylem parenchyma by aerial-wound inoculation of both resistant and susceptible plants. The hypertrophy of xylem parenchyma that Linford (*9*) observed when he injected fungal filtrates through cut petioles of susceptible plants was not found in any of the diseased resistant plants. Another factor which might have accounted for the increased diameter of the wilt-

infected plants was the retardation in the development of the schizogenous lacunae.

Susceptible plants grown in sand at 27° usually exhibited a water soaking of the tissues in the first and second internodes when completely wilted. In such plants the fungus was found abundantly throughout the cortex and stele of these internodes (pl. 4, *C*). Such water soaking was rare at 24° and below, and microscopic examination of such tissues indicated a situation comparable to that described by Linford (*7*) in which the fungus was confined to the stele. The fungus was found only sparingly in the xylem of completely wilted susceptible plants grown at 15°, and the aerial symptoms of such plants closely resembled those of the completely wilted resistant plants grown at 27° and 30° (fig. 6). A transection of such a plant is compared in plate 2 with those of a completely wilted resistant plant at 27°, and corresponding healthy plants. The hyphae were found in only two xylem elements of the susceptible plants, but both these vessels were crowded (pl. 3, *B*). The fungus occurred in more than two xylem vessels in the resistant plant, but hyphal invasion of any one vessel was limited to one or two hyphae (pl. 3, *A*), and deposition of granular and gumlike material was more prevalent. Microscopic examination of other completely wilted plants grown at the higher sand temperatures indicated a greater frequency of occluded vessels in the resistant variety. Such occlusions were not necessarily associated with invaded cells, nor did they appear to hinder the fungus in all cases (pl. 3, *A;* pl. 4, *D*). The protoxylem vessels appeared to be more frequently occluded than other vessels. Occasionally, cells were found in which the hyphae appeared enlarged and somewhat granulated in contrast to the smaller and more normal-appearing hyphae in the invaded cells of susceptible plants.

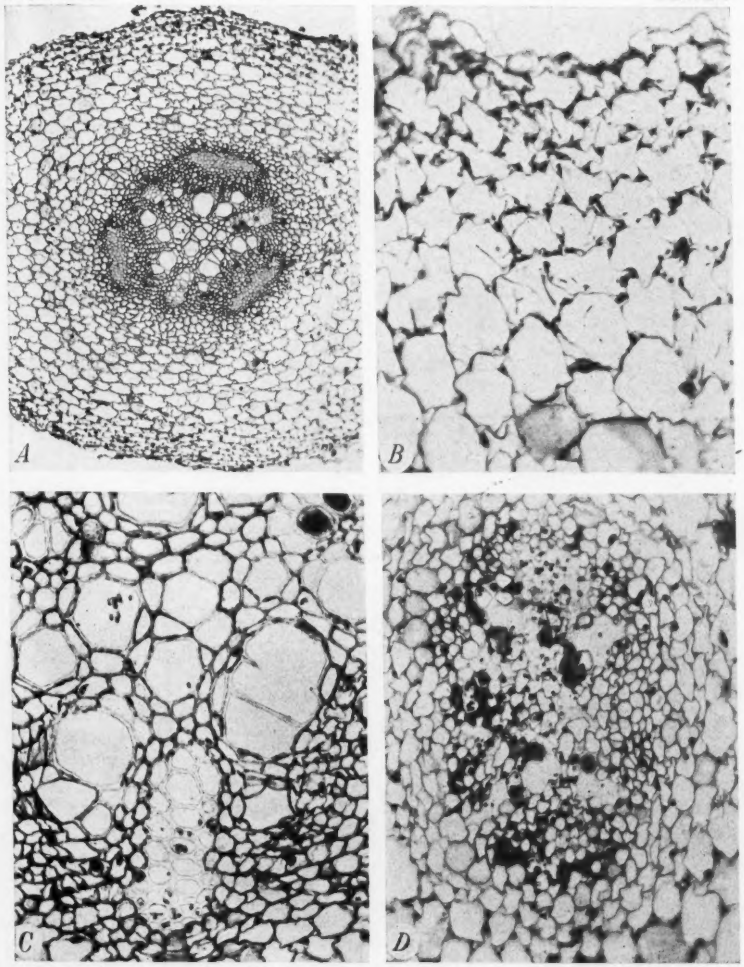
CROSS-INOCULATION STUDIES

The apparent break-down of wilt resistance in garden pea when plants were grown in inoculated sand supplied with a weak nutrient at high temperatures raised the question whether the host range of this organism could be extended under such conditions to hosts of other equally specialized vascular fusaria. Cross-inoculation experiments were therefore made with this organism, the cabbage yellows organism, and the tomato wilt organism, *F. oxysporum* Schlecht f. *lycopersici* (Sacc.) S. & H. (*F. bulbigenum* var. *lycopersici* (Brushi) Wr. & R.). This seemed especially pertinent in view of the recent work of Armstrong et al. (*2*) and Smith and Shaw (*15*), wherein definite wilting of supposedly immune hosts was obtained by inoculation with various vascular fusaria under special methods of nutrition and inoculation.

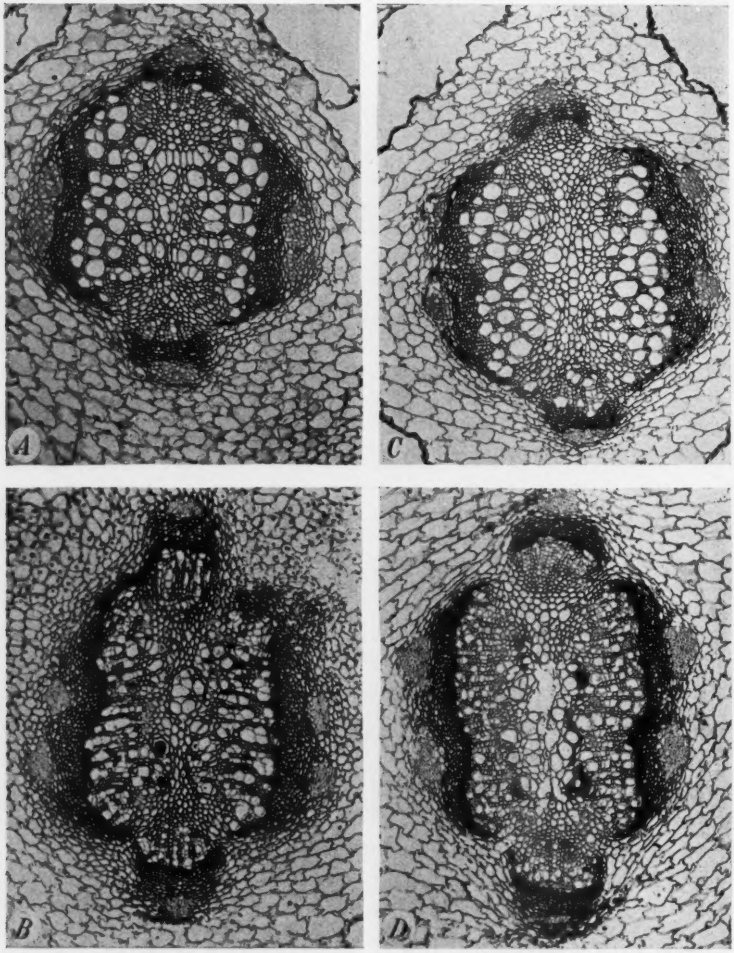
Two varieties of each host were used, one resistant and one susceptible to the respective organism which was known to be specialized to that host. The following varieties were included:

Host:	*Resistant variety*	*Susceptible variety*
Tomato	Red Currant [1]	John Baer.
Cabbage	Jersey Queen	Round Dutch.
Pea	Wisconsin Perfection	Davis Perfection

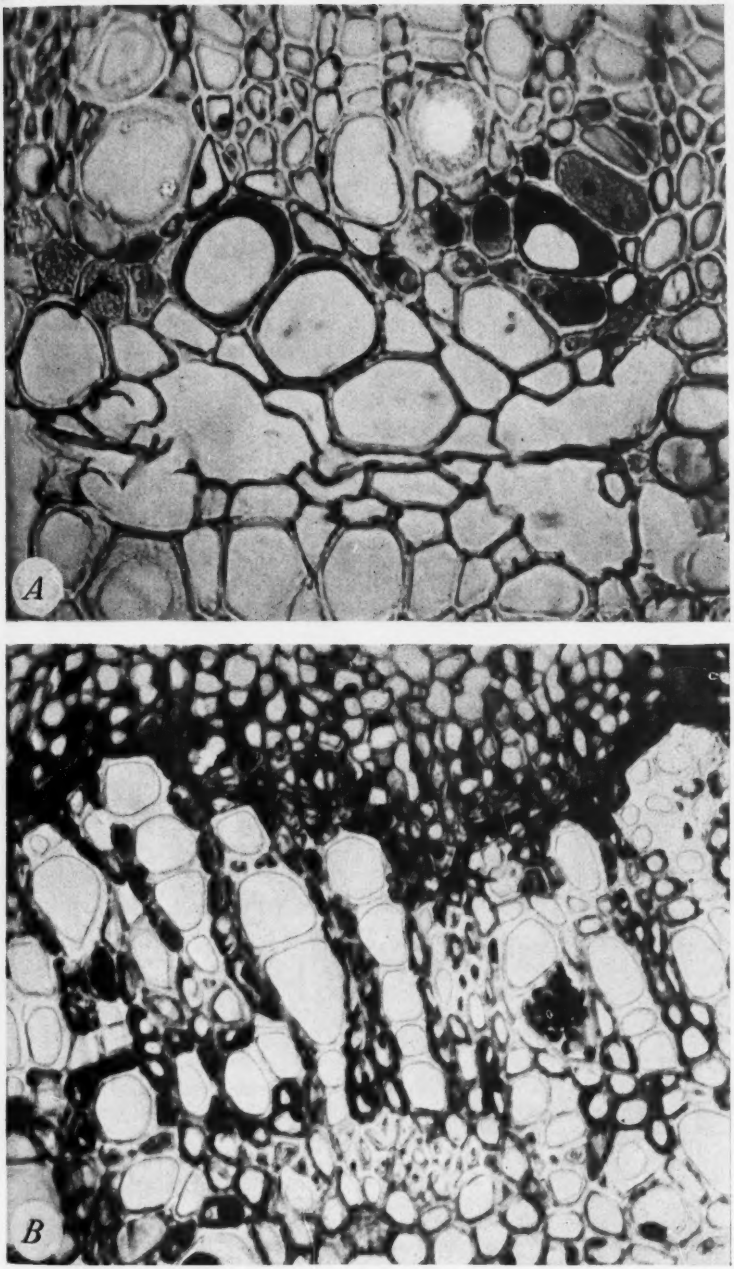
[1] A highly resistant strain of this species (*Lycopersicon pimpinellifolium* Mill.) was used.



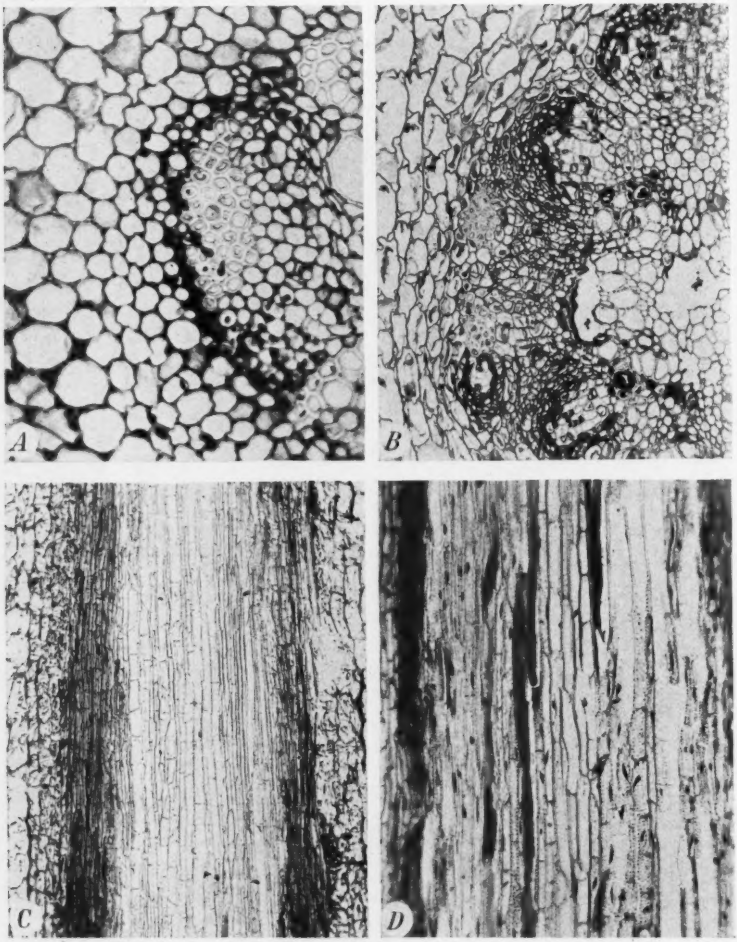
Transections of the roots of Wisconsin Perfection (resistant) peas. *A*, Penetration of the outer cortical cells and stele, ×105; *B*, enlarged portion of outer cortex in *A*, showing intra- and inter-cellular penetration, × 433; *C*, enlarged portion of stele in *A*, showing hyphae in metaxylem and protoxylem arm and also occlusions in some of the vessels, × 433; *D*, lateral root showing hyphae in cortex and stele and occlusions in some of the vessels, × 433.



A to *C*, Transections of the first internodes of the plants illustrated in figure 7 to show similarity in host-parasite relations in resistant and susceptible plants. All × 105. *A*, Healthy Davis Perfection pea (susceptible) grown at 15° C. sand temperature; note the development of schizogenous lacunae and normal cambial activity. *B*, Wilted Davis Perfection pea grown at 15°; only two vessels are invaded but hyphae are abundant in those two; note the increased cambial activity and development of secondary xylem and phloem, especially in the plane of the polar bundles; the abundance of nuclei is the result of the development of adventitious roots at a lower level. See further enlargement in plate 3, *B*. *C*, Healthy Wisconsin Perfection (resistant) grown at 27°; note similarity to *A*. *D*, Wilted Wisconsin Perfection grown at 27°; compare with *B* for abnormal development of stelar tissues. Only one or two hyphae were contained in each of the invaded vessels; note the greater number of occluded and granulated cells. See further enlargement in plate 3, *A*.



A, Enlarged portion of stele of wilted Wisconsin Perfection (resistant) pea plant grown at 27°, illustrated in plate 2, *D*, showing rather sparse occurrence of the fungus and abundance of granular and gumlike depositions in the cells; note the two hyphal ends in the granulated cell to the right and the intercellular penetration of the hypha from a clear cell to an adjacent granulated cell. *B*, Enlarged portion of stele of wilted Davis Perfection (susceptible) pea plant grown at 15°, illustrated in plate 2, *B*, showing abundance of hyphae in the only two invaded vessels.



A, Transection of root of Wisconsin Perfection (resistant) plant showing penetration of hyphae in the cortex, phloem, and pericycle, as well as in some of the phloem fibers, × 433. *B*, Transection of the first internode of a completely wilted Wisconsin Perfection plant at 30° C. showing an extreme reaction to infection; the fungus was present in the vessels, but not extensively. Note the cellular occlusions and the proliferation of vessels in the xylem region adjacent to the phloem fiber bundle, × 433. *C*, Longitudinal section of the first internode of a completely wilted Davis Perfection (susceptible) plant at 27°, showing an abundance of hyphae in the cortex and cambial region, as well as in the stele; note the apparent absence of occlusions. *D*, Longitudinal section of the first internode of Wisconsin Perfection plant at 27°, showing considerable plugging of the vessels, especially in the protoxylem; compare with C.

The sand supporting the growth of these plants was held at 27° to 28° C., and supplied with the 0.1H concentration of nutrient solution. Seed of the pea varieties and transplants of the tomato and cabbage varieties were planted directly in the artificially infested sand. For the sake of compatibility with respect to any predisposing influence, the seed for the cabbage and tomato transplants was sown in sterilized sand supplied with the 0.1 H nutrient solution and maintained at approximately 28°. The crocks were planted with eight pea seeds and five to six transplants of cabbage and tomato. The inoculum was prepared as previously described for the pea wilt organism. The medium proved equally suitable for all three pathogens; the inocula consisted entirely of microconidia and mycelial fragments. All crocks were planted in duplicate. Notes were taken on the initial appearance of the disease, full symptom expression, and death of the plants.

The results indicated that all three pathogens were specific to their respective hosts under the conditions of this experiment. Davis Perfection (susceptible), inoculated with the pea wilt organism, succumbed to the disease 24 days after planting, while Wisconsin Perfection (resistant) exhibited the slow disease development characteristic of these plants under the conditions of low nutrient and high temperature. Plants of John Bear tomato (susceptible) inoculated with the tomato wilt fungus, and Round Dutch cabbage (susceptible) inoculated with the cabbage yellows organism, were dead at the end of 17 days. No visible disease symptoms occurred on Red Currant tomato in sand artificially infested with the tomato wilt organism. Jersey Queen cabbage (resistant), in the cabbage yellows sand, exhibited a severe expression of the atypical symptoms described by Walker and Smith (*22*); some plants died. Moreover, none of the hosts grown in sand infested with their respective nonspecific parasites was different in external appearance from the corresponding control plants.

Nodal platings of the surviving plants indicated that the pathogens might have penetrated the nonspecific hosts even though they did not give rise to any symptom expression. This was especially characteristic of the resistant and susceptible varieties of pea. Platings were made of the root, and of the second, fourth, and sixth nodes of the stem. The respective fungus was recovered from the second node of each plant and from the root and the third or fourth node of a few plants. The constant location of the organism in the second node suggested that penetration occurred through the region of cotyledonary attachment. The significance of these findings depends upon further studies and on re-cross inoculations with the fungi recovered from these plants. Nevertheless, the results indicate that of the three species only the pea wilt organism can produce symptoms in either the susceptible or the resistant variety of pea.

DISCUSSION

The fusarium wilt of pea has been of considerable biological interest, principally because of its several points of contrast with other related vascular fusarioses. Notable among these features have been the temperature relations, the symptoms, and the failure in attempts to

break down the inherent and clear-cut resistance of some varieties. The results presented in this paper show that, when the soil factor is eliminated by growing peas in sterile sand artificially infested with a pure culture of the pea wilt organism, disease development increases directly with the temperature of the sand, up to 27°. Weight of the mycelium of the fungus grown in the nutrient solution with added carbohydrate, is also greatest at that temperature. Thus the optimum temperature of pea wilt is essentially similar to that of tomato wilt, cabbage yellows, and near wilt of pea. The extreme departure from the disease-soil-temperature curve might have been explained on the basis of a new physiologic form or race of *F. oxysporum* f. *pisi* race 1 were it not for the fact that a parallel series showed that in plants growing in soil disease development was neither as rapid nor as severe at 27°. Linford (*7*) suggested that the retardation in disease development at soil temperatures most favorable for the growth of the fungus be explained on the basis of resistance induced in the host by the high temperature. This view hardly seems tenable in the light of the present results, unless one assumes that the effects of temperature on plants in soil are remarkably different from those in sand. One possible explanation of the difference in reaction in soil and sand might be found in a study of the biological antagonism to the pea wilt organism in the soil at high temperatures. This suggestion does not seem too remote, when one considers the difficulty encountered by Walker and Snyder (*23*) in establishing the pea wilt fungus in certain types of soils, in contrast to the ease with which the cabbage yellows organism was established.

When plants were grown in sand held at 21° and supplied with nutrients at different concentration levels during that time of year when the periods of sunlight were fairly long, it was observed that the severity of disease development in susceptible plants decreased with a rise in the nutrient concentration level. Under the same conditions, the most severe development occurred in the resistant variety at 0.1H, very little occurred at 1H, and no evidence of disease was noted at 3H and 5H. These results are in agreement with those obtained by Smith and Walker (*14*) in a study of the relation of nutrient concentration to infection and severity of aphanomyces root rot of pea. During the middle of the winter, when the days were short and cloudy, the resistant plants reacted similarly but no significant differences were obtained in the wilt indices of susceptible plants at the various concentration levels. No explanation of this discrepancy can be given at the present time, but it would appear that a study of the effect of the duration and intensity of light on the development of the disease under the conditions of these experiments might yield important results.

When plants were grown at 27°, the results were altogether different. Instead of very little disease development occurring at the 5H nutrient, the disease was most severe at that level and no difference was observed between the susceptible and resistant varieties. Resistant plants succumbing under these conditions resembled the susceptible plants in the 0.1H nutrient level at 27°, except that the plants reached the complete-wilt stage in a much shorter time at the highest level. This occurred in both experiments, regardless of any possible effect of the light period. Cortical decay was very severe in the plants

at the high nutrient concentration level, and the abundance of fungal growth and its accompanying toxic action undoubtedly contributed to the rapid wilting.

It will be noted that Linford (*9*) observed extensive cortical decay in susceptible plants grown in wilt-infested soil under pure culture conditions. Moreover, the artificially infested sand used in the present investigation very closely approximated a pure culture condition, and in addition, the biological factors of the soil were eliminated. Anderson and Walker (*1*) also observed extensive cortical decay in the root hypocotyl of the homozygous resistant cabbage, Wisconsin Ballhead, when grown in yellows-infested soil at high temperatures. Thus it is to be seen that two diseases, both considered to be strictly vascular and controlled by clear-cut resistance under natural conditions, can be changed in their host-parasite relations by altering the conditions under which disease development occurs. Other fusarial wilts, such as watermelon wilt, muskmelon wilt, and fusarium yellows of celery, considered to be less strictly vascular wilts, develop a cortical decay in the roots under less drastic changes in environal conditions. Nelson, Coons, and Cochran (*11*) observed extensive decay of celery roots in the field, expecially when temperatures were quite high, whereas cortical decay and seedling rot occurred in plants affected with watermelon and muskmelon wilt at relatively low soil temperatures (*6*, *12*, *13*, *24*).

The thermal and nutritional relations of the host, the parasite, and the interaction of the two may be seen to have an influence on the type and extent of disease development. The extension of the pathogen in the vascular system of the host was greatest at 24° sand temperature and next most rapid at 21°. Swelling of the first stem internode following infection, due to vascular proliferation and to the lack of schizogenous lacunae, was greatest at 21°. These phases of the disease reach their peak, therefore, at temperatures close to that of optimum growth of the host. Cortical decay was greatest at 27° and 30°, points beyond that of best growth of the host but coincident with that for most rapid development of the pathogen. If this is compared with the thermal relations of watermelon wilt (*12*, *13*, *24*) it will be seen that the necrotic phase in the latter occurs chiefly at the lower temperatures which are least favorable to the host, while the typical wilt symptoms and vascular invasion come at the higher temperatures which are favorable to the host as well as to the parasite. It is thus very apparent that the influence of temperature on development of vascular wilts is not a simple one. The influence of nutrition throws much light on this question and furthermore this is shown to be an important interacting factor. It is clear that with light and temperature conditions favorable to growth of the pea plant an increase in available nutrients results in suppression of disease development in both resistant and susceptible varieties. However, at a temperature most favorable to the pathogen and less favorable to the host high nutrient levels have an opposite effect in that they promote cortical necrosis and rapid wilt development.

All attempts by other workers to break down resistance to pea wilt by such practices as severe root pruning and wounding of established plants or transplants has failed (*7*, *9*, *19*, *21*). Most of this work was done in soil or on soil-extract agar at about 21° C., which was con-

sidered to be the optimum temperature for disease development. Occasionally evidence of slight invasion of the root tips of resistant plants was found, but, in contrast to the susceptible plants where the organism progressed from the root tip through to the stele and involved very little cortical decay, further progress ceased at this point. As a result, resistance was suggested to be located in the undifferentiated tissues of the young roots. In this investigation it was found that, as in cabbage yellows (*1*, *22*), resistance to pea wilt was not retained at high sand temperatures. Resistant plants developed above-ground symptoms at high temperatures quite similar to susceptible plants at the lower sand temperatures of 15° and 18°. Coincident with slow wilt development in susceptible plants at 15° there was a very sparse invasion of the vascular elements and no cortical decay. In resistant plants grown at sand temperatures of 27° and 30° hyphal accumulation was also very sparse, but the internal reaction in such plants was not exactly the same as that of susceptible plants at 15°. In susceptible plants growing at 27° in sand supplied with either the 0.1H or the 5H nutrient solution and in the resistant plants supplied with the 5H at the same temperature very rapid wilting usually occurred. In such plants wilting simulated that in the severe development of tomato wilt. Cortical decay was extensive throughout the root system and the lower internodes, but wilting did not result from a collapse of the water-soaked lower internodes, since they were the last to collapse, indicating that the rapid wilting from the tip down might have been due to an abundance of toxin produced by the fungus. Hyphae were found in abundance throughout the cortex and stele of these lower internodes, but further extension through the xylem and cortex seemed to stop rather abruptly. From these observations it would appear that the severity of disease expression was related to the amount of hyphal invasion. Additional evidence in support of this suggestion was brought out in the nodal isolations of completely wilted plants grown at the various sand temperatures.

In addition to the very close similarity in symptoms between the susceptible plants at the low temperature and resistant plants at the high temperatures under conditions of low nutrition, microscopic examinations of the epicotyledonary tissues indicated that hyphal invasion of the stele was very sparse. Evidences of disturbances within the susceptible host were only rarely found, even in those rapidly wilted at the higher temperatures. Depositions of gumlike and granular materials were quite common in the stelar tissues of the epicotyl of resistant plants, and occurred to a lesser extent in the root. Almost all of the invaded vessels in resistant plants contained only one or two hyphal strands in the epicotyledonary tissues, in contrast to the root tissues where invasion of both cortex and stele was quite abundant. This fact, together with the pronounced host reaction, would seem to indicate that, even though well established within the root system, the organism progressed with difficulty through the resistant stem tissue. The evidence obtained from these studies seems to indicate that resistance to pea wilt is not necessarily located in the undifferentiated tissues of the root, for even though the organism invades the entire root system, it is still hindered in its progress through the stelar tissues of the stem. The nature of this hindrance would in a large part determine the nature of resistance.

The results obtained in the cross-inoculation studies validate those obtained with respect to the break-down in resistance to pea wilt under conditions of very low nutrition. Cross inoculations of susceptible and resistant varieties of tomato, cabbage, and pea with their respective fusarial wilt pathogens failed to alter their specificity, although the nodal isolation of these plants indicated that the fungi had penetrated the nonspecific hosts. The extent of penetration, as measured by the nodal isolations, was no greater in the resistant pea variety inoculated with the pea wilt organism than in the same variety inoculated with the cabbage yellows or tomato wilt pathogens. The presence of the pea wilt fungus in the plant, however, produced definite wilt symptoms; whereas no symptons were observed on the same variety infected with the other two pathogens. Armstrong et al. (*2*) studied cross inoculations of various crop plants with their respective fusarial wilt pathogens and found that some were successful with respect to symptons; others were not, although in many cases the fungus was recovered. The three pathogens used in the studies made by the writers were not included in Armstrong's work. These results, although not conclusive, suggest the possibility that a given fusarium pathogen may become established in a nonspecific host without producing symptoms, either because of its inability to produce a wilt-inducing toxin in that particular plant or because of the ability of the plant to tolerate any toxin produced.

SUMMARY

The investigations comprised a study of the influence of temperature and nutrition upon the development of fusarium wilt of pea (*Fusarium oxysporum* f. *pisi* race 1) in a susceptible and a resistant variety.

Under conditions of controlled nutrition and temperature in sand artificially infested by means of a suspension of microconidia and hyphal fragments, the optimum temperature for disease development was found to be 27° and 30° C. instead of 21° as previously observed in infested soil. Air temperature had relatively little influence on disease development. The fungus grew best at 28° when cultured in the same nutrient solution used in the sand culture of the host to which carbohydrate had been added.

The severity of disease development in the susceptible plants appeared to be directly proportional to the temperature, within the range studied. Symptons varied from a very slow wilt characterized by leaf necrosis and abscission at the low sand temperature to a very rapid wilting at the highest temperature studied. Cortical decay occured to some extent at all temperatures but was most severe and involved the lower internodes only at the optimum temperature. The greatest progress of the fungus up the stem occured in those plants growing at the 24° and 21° sand temperatures. The lower internodes of diseased plants were significantly greater in diameter than the corresponding control plants.

The development of the disease in the homozygous resistant plants varied from off color and very slight incurving of the lower stipules and leaflets at the low temperatures and low nutrient concentration to severe wilting at the very highest concentration and optimum temperature. At high temperatures and low nutrient concentration diseased

resistant plants were similar to diseased susceptible plants at the low temperature.

The disease development in resistant and susceptible plants at various concentrations of the nutrient solution was found to depend upon the sand temperatures. Four nutrient solutions, differing only in total salt concentration and designated, in order of concentration, as 0.1H, 1H, 3H, and 5H were used. At 21°, the time required for the appearance of complete wilting in susceptible plants varied directly with the concentration of the nutrient solution; in resistant plants disease development was most severe in the 0.1H solution, and none occurred at the higher concentrations. At 27°, disease development was most severe in the 5H solutions, and no differences in symptoms were observed between susceptible and resistant plants. The least amount of disease development in both resistant and susceptible plants occurred in the 1H solution; that at the 3H level was quite variable. In the 0.1H solution, resistant plants developed a very severe case of slow wilting, while susceptible plants approached the rapid wilting occurrent in the 5H solution.

No differences were found in the growth rates of the fungus on agar media made from the nutrient solutions employed in the sand culture with added carbohydrate that could account for the differences observed in the disease development of plants growing in the different solutions at any given temperature.

Microscopical examination of diseased resistant plants indicated extensive cortical and stelar penetration of the roots. Stelar penetration was observed in sections taken from the first and second internodes; nodal isolations indicated still higher advances. More pronounced host reaction to the fungus, in the form of granular and gum-like depositions and scarcity of hyphal strands, was observed in resistant plants at the high temperatures than in susceptible plants at either high or low temperature.

Cross inoculations of susceptible and resistant varieties of tomato, pea, and cabbage with their respective fusarial wilt pathogens under the conditions of high temperatures and low nutrient sand culture indicated that the break-down in pea wilt resistance could be accomplished only with the pea wilt organism.

LITERATURE CITED

(1) Anderson, M. E., and Walker, J. C.
1935. Histological studies of Wisconsin Hollander and Wisconsin Ballhead cabbage in relation to resistance to yellows. Jour. Agr. Res. 50: 823–836, illus.

(2) Armstrong, G. M., Shanor, L., Bennett, C. C., and Hawkins, B. S.
1941. Some Fusarium wilt organisms. (Abstract of Paper) Phytopathology 31: 1–2.

(3) Bohn, G. W., and Tucker, C. M.
1940. Studies on Fusarium wilt of tomato: I. Immunity in Lycopersicon pimpinellifolium Mill. and its inheritance in hybrids. Mo. Agr. Expt. Sta. Res. Bul. 311, 82 pp., illus.

(4) Hoagland, D. R., and Snyder, W. C.
1934. Nutrition of strawberry plant under controlled conditions: (a) effects of deficiencies of boron and certain other elements: (b) susceptibility to injury from sodium salts. Amer. Soc. Hort. Sci. Proc. (1933) 29: 288–294, illus.

(5) JOHANSEN, D. A.
1940. PLANT MICROTECHNIQUE. 523 pp., illus. New York and London.
(6) LEACH, J. G., and CURRENCE, T. M.
1938. FUSARIUM WILT OF MUSKMELONS IN MINNESOTA. Minn. Agr. Expt. Sta. Tech. Bul. 129, 32 pp., illus.
(7) LINFORD, M. B.
1928. A FUSARIUM WILT OF PEAS IN WISCONSIN. Wis. Agr. Expt. Sta. Res. Bul. 85, 44 pp., illus.
(8) ———
1931. TRANSPIRATIONAL HISTORY AS A KEY TO THE NATURE OF WILTING IN THE FUSARIUM WILT OF PEAS. Phytopathology 21: 791–796, illus.
(9) ———
1931. STUDIES OF PATHOGENESIS AND RESISTANCE IN PEA WILT CAUSED BY FUSARIUM ORTHOCERAS VAR. PISI. Phytopathology 21: 797–826, illus.
(10) ———
1931. WOUND INOCULATION IN RELATION TO RESISTANCE IN THE FUSARIUM WILT OF PEAS. Phytopathology 21: 827–833, illus.
(11) NELSON, R., COONS, G. H., and COCHRAN, L. C.
1937. THE FUSARIUM YELLOWS DISEASE OF CELERY (APIUM GRAVEOLENS L. VAR. DULCE D. C.). Mich. Agr. Expt. Sta. Tech. Bul. 155, 74 pp., illus.
(12) PORTER, D. R.
1928. INFECTION STUDIES WITH WATERMELON WILT CAUSED BY FUSARIUM NIVEUM E. F. S. Iowa Agr. Expt. Sta. Res. Bul. 112, pp. [347]–368, illus.
(13) ——— and MELHUS, I. E.
1932. THE PATHOGENICITY OF FUSARIUM NIVEUM (EFS.) AND THE DEVELOPMENT OF WILT RESISTANT STRAINS OF CITRULLUS VULGARIS (SCHRAD.). Iowa Agr. Expt. Sta. Res. Bul. 149, pp. [121]–184, illus.
(14) SMITH, P. G., and WALKER, J. C.
1941. CERTAIN ENVIRONAL AND NUTRITIONAL FACTORS AFFECTING APHANOMYCES ROOT ROT OF GARDEN PEA. Jour. Agr. Res. 63: 1–20, illus.
(15) SMITH, T. E., and SHAW, K. J.
1941. SUCCESSFUL CROSS-INOCULATIONS WITH FUSARIUM FROM TOBACCO AND SWEET POTATO. (Abstract of Paper) Phytopathology 31: 21.
(16) SNYDER, W. C.
1933. VARIABILITY IN THE PEA-WILT ORGANISM, FUSARIUM ORTHOCERAS VAR. PISI. Jour. Agr. Res. 47: 65–88, illus.
(17) VIRGIN, W. J., and WALKER, J. C.
1939. RELATION OF TEMPERATURE AND MOISTURE TO NEAR-WILT OF PEA. Jour. Agr. Res. 59: 591–600, illus.
(18) ——— and WALKER, J. C.
1940. RELATION OF THE NEAR-WILT FUNGUS TO THE PEA PLANT. Jour. Agr. Res. 60: 241–248, illus.
(19) WADE, B. L.
1929. INHERITANCE OF FUSARIUM WILT RESISTANCE IN CANNING PEAS. Wis. Agr. Expt. Sta. Res. Bul. 97, 32 pp., illus.
(20) WALKER, J. C.
1930. INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE. Jour. Agr. Res. 40: 721–745, illus.
(21) ———
1935. A STUDY OF RESISTANCE TO FUSARIUM WILT IN ALASKA PEAS. Amer. Jour. Bot. 22: 849–857.
(22) ——— and SMITH, R.
1930. EFFECT OF ENVIRONMENTAL FACTORS UPON THE RESISTANCE OF CABBAGE TO YELLOWS. Jour. Agr. Res. 41: 1–15, illus.
(23) ——— and SNYDER, W. C.
1933. PEA WILT AND ROOT ROTS. Wis. Agr. Expt. Sta. Bull. 424, 16 pp., illus.

(24) WILSON, J. J.
1936. THE PATHOLOGICAL RELATIONSHIP BETWEEN THE HOST AND PARASITE IN VARIETIES AND STRAINS OF WATERMELONS RESISTANT TO FUSARIUM NIVEUM E. F. S. Iowa Agr. Expt. Sta. Res. Bul. 195, pp. [105]–152, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1942

INFLUENCE OF HEREDITY AND OTHER FACTORS ON 180-DAY WEIGHT IN POLAND CHINA SWINE [1]

By J. A. WHATLEY, Jr.

Formerly *research fellow, Iowa Agricultural Experiment Station; now cooperative agent, Bureau of Animal Industry, United States Department of Agriculture, and Assistant Animal Husbandman, Oklahoma Agricultural Experiment Station*

INTRODUCTION

Rate of gain to market weight is one of the important factors in economical swine production, since rapid gains cause lower maintenance costs and presumably indicate more vitality and a higher degree of health. If breeders are to make the best use of selection and various breeding systems in improving rate of gain in their herds, it is important to know something of the heritability of individual differences in this character. This paper presents a study of the effect of certain environmental factors on weight at 180 days of age and estimates of what part of the variance is hereditary as determined by the resemblance between certain kinds of relatives.

Few quantitative studies have been made of the influence of heredity on growth rate in swine. Lush and his coworkers (*5*)[2] concluded that 6 percent of the total variance in birth weight was attributable to differences in the heredity of the pigs, 47 percent to the environment common to litter mates, and 47 percent to the environment not common to litter mates. A part of the environmental effects common to litter mates may of course have been due to hereditary characteristics of their dams. From the correlations between certain relatives, Bywaters (*1*) concluded that one-fifth of the variance in weaning weight was determined by the heredity of the pig. Lush (*4*) studied Danish testing-station data and concluded that one-fifth of the variance in daily gain while the pigs were on test could be attributed to additive genetic effects. Smith and Donald (*6*) observed the correlation between the growth rates of litter mates to be 0.3 and 0.5 for litter sizes of 8 and 9, respectively. They concluded that at least one-fifth of the individual variance in growth rate was due to additive genetic factors.

MATERIAL AND METHODS

The data for the present investigation were taken from an inbreeding experiment with Poland China swine begun at the Iowa Agricultural

[1] Received for publication October 23, 1941. Journal Paper No. J-917 of the Iowa Agricultural Experiment Station. Project No. 32. The studies reported here were made under the guidance of Dr. Jay L. Lush. The most recent of the data were collected as part of the cooperative work of the U. S. Regional Swine Breeding Laboratory.

[2] Italic numbers in parentheses refer to Literature Cited, p. 263.

478749—42——3

Experiment Station in 1930. A brief history of the foundation animals has been given by Bywaters (*1*). The foundation stock consisted of 27 sows and 3 boars purchased from various breeders. Some of the sows were already bred when purchased and through these the offspring of 3 more boars were included in the foundation stock. No outside blood was introduced into the herd after 1930. Most of the foundation animals were not inbred. The 12 which were inbred had inbreeding coefficients of less than 4 percent. All inbreeding coefficients were computed on pedigrees traced to 1925 as a base date.

Each season the general breeding plan was to use four boars and to allot the sows so that each boar was mated to a group approximately equal to the other groups in average age, past productiveness, type, and in relationship to the boar to which they were bred. In the fall of 1937 the herd became a part of the Regional Swine Breeding Laboratory herd, and the original four-sire herd was split into several noninterbreeding lines within which there were different rates of inbreeding. However, the present study includes only one pig crop after the separation of lines, and the lines had not yet diverged far enough from the original four-sire herd to be materially different from each other.

During the gestation and suckling period all sows were fed and managed in the same manner. Pigs were weaned at 60 days in the first years of the experiment, but beginning with the fall of 1937, the weaning age was advanced to 56 days. After weaning, the pigs were fed good rations and were managed in accordance with good practices, which should have permitted them to show their capabilities. Although the pigs were not all fed and handled in exactly the same way, the different treatments were all reasonably good and the average 180-day weight of the groups on different treatments varied little. When the data were corrected for such treatment differences as did exist, the analyses gave results in such close agreement with the uncorrected data that corrections for treatment differences were not considered necessary for the present analysis.

The data covered 5 spring farrowing seasons (from 1934 through 1938) and 3 fall farrowing seasons (1934, 1935, and 1937). The 1,394 pigs produced during this period were in 267 litters, which were out of 151 sows and by 23 boars.

Weight at 180 days, rather than at some other age, was selected as the measure of gain in this study because at this age the faster growing pigs should be approaching a desirable market weight of 200 to 225 pounds. For pigs weighed a few days older or younger than 180 days, the 180-day weight was estimated by multiplying the actual weight by $\frac{(180-60)}{(\text{actual age}-60)}$. This adjustment was derived from the intercepts of straight lines fitted to several growth curves as was suggested by Bywaters and Willham (*2*). A later study [3] revealed that an intercept of 60 days gave more consistent results than the intercept of 65 days suggested by Bywaters and Willham. The data were analyzed chiefly by means of correlation, regression, analysis of variance, and covariance (*7*).

[3] WHATLEY, J. A., JR. A METHOD FOR COMPARING THE GROWTH RATES OF PIGS WEIGHED AT DIFFERENT AGES. Unpublished thesis. Iowa State College, Ames, Iowa. 1937.

INFLUENCE OF DIFFERENT FACTORS AFFECTING 180-DAY WEIGHT

INFLUENCE OF INBREEDING

The average inbreeding of all pigs was 14.7 percent, which is slightly higher than the inbreeding resulting from one generation of half-brother and sister mating (12.5 percent). The average weight and average inbreeding for each farrowing season are plotted in figure 1.

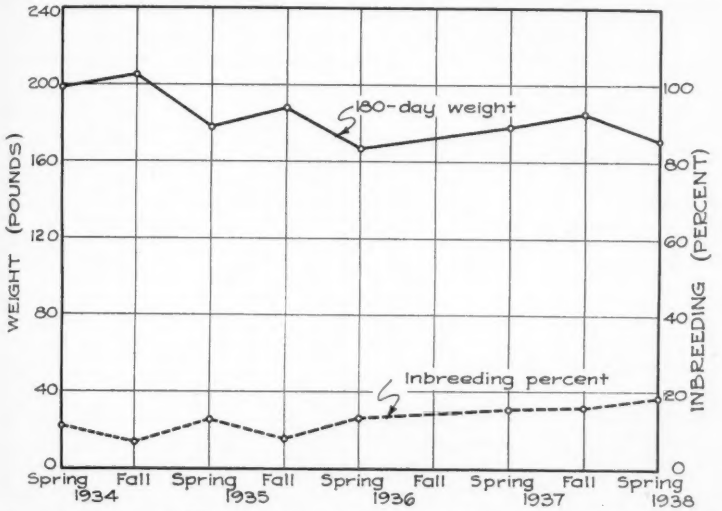


FIGURE 1.—Average 180-day weight and percent of inbreeding by season and year.

The average weight decreased slightly during the period studied, while the inbreeding gradually increased. The inbreeding was low in the fall seasons of 1934 and 1935, because at that time the policy was to produce fall pigs only from sows at least 2½ years old, and these naturally were less closely related to the boars than were sows bred for spring farrowing, about half of which were gilts. The average inbreeding of the sires and dams of the pigs was 9.3 percent.

A correlation of -0.17 ± 0.03 [4] was observed between the inbreeding and the 180-day weight of pigs born in the same farrowing season. The corresponding regression coefficient was 0.76 pound decline in 180-day weight for each 1 percent increase in inbreeding. Table 1 shows the average 180-day weights when grouped according to the percentage of inbreeding. Except in the last two classes there was a noticeable decrease in weight with an increase in inbreeding. The sharp rise in weight in the two classes with the highest inbreeding may have been due to sampling errors, since the number of pigs in these classes was small.

[4] The figures after the ± signs in this paper are standard errors. For correlations they were computed according to the formula $\frac{1-r^2}{\sqrt{n-2}}$, which seemed a good enough approximation for correlations no larger than these.

When each litter was treated as a unit (rather than each pig as above), the correlation between 180-day weight and inbreeding was -0.19 ± 0.06. The effect of the dam's inbreeding was negligible in these data, but as this mild inbreeding system was really just getting well under way, none of the dams were highly inbred.

INFLUENCE OF SEASON AND OF YEAR

The average 180-day weight of all pigs was 180.3 pounds and the standard deviation was 31.3 pounds. Figure 2 shows the distribution of the weights of the 1,394 pigs.

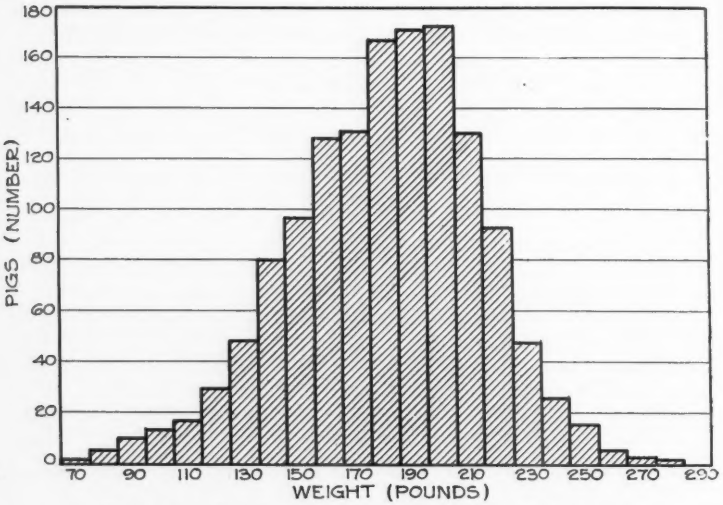


FIGURE 2.—Distribution of 180-day weights of 1,394 pigs.

The average weight of the 1,190 spring-farrowed pigs was 178 pounds as compared with an average weight of 194 pounds for the 204 fall-farrowed pigs. The difference of 16 pounds was highly significant statistically and is large enough to be economically important. Its importance as compared to other sources of variation is shown by the fact that the mean variance within pairs of pigs, one of which was born in the fall and the other in the spring, was 12.8 percent larger than the mean variance between pigs born in the same season.

TABLE 1.—*Regression of 180-day weight on percent of inbreeding*

Inbreeding (percent)	Litters	Pigs	180-day weight	Inbreeding (percent)	Litters	Pigs	180-day weight
	Number	*Number*	*Pounds*		*Number*	*Number*	*Pounds*
3	20	109	199	33	20	92	159
9	88	473	187	39	4	14	192
15	77	398	177	45	1	4	178
21	46	243	177				
27	11	61	166	Total or average	267	1,394	180

In measuring the effect of year-to-year changes on 180-day weight only spring-farrowed pigs were considered, since fall pigs were not produced in all years. Among the spring-farrowed pigs the mean square between years was significantly larger than the mean square between litters farrowed in the same year. The variance between pigs born in different years averaged 11 percent higher than that between pigs born in the same year. This expresses quantitatively, for comparison with other sources of variance, the importance of the year-to-year variations in mean weights which are shown graphically in figure 1. It seems likely that a part of this year-to-year variation results from the time trend toward more intense inbreeding.

INFLUENCE OF DAM'S AGE

The influence of age of dam on the weight of pigs at 180 days was not statistically significant, although the figures in table 2, if accepted at face value, do indicate that pigs from older dams were slightly heavier. The superior nursing ability of the older dams might account for this slight difference (if more extensive data prove its reality), but also the older dams were selected in part on the performance of their previous litters and this might account for some such difference.

TABLE 2.—*Average 180-day weights of spring and fall pigs from dams of different ages*

Spring			Fall		
Age of dam (years)	Pigs	Average 180-day weight	Age of dam (years)	Pigs	Average 180-day weight
	Number	*Pounds*		*Number*	*Pounds*
1	624	174	1.5	35	192
2	293	180	2.5	99	195
3	136	182	3.5	27	189
4	77	189	4.5	27	193
5	49	182	5.5	16	206
6	11	164			
Total	1,190	178		204	194

INFLUENCE OF SEX

Gilts weighed about 4 percent less than barrows and boars at 180 days, and this difference was highly significant statistically. The average weight of the 694 males (620 barrows and 74 boars) was 184 pounds and the average weight of the 700 gilts was 177 pounds. The variance between pigs of unlike sex was about 2 percent larger than the variance between pigs of the same sex.

INFLUENCE OF BIRTH WEIGHT AND WEANING WEIGHT

The usefulness of birth weight and weaning weight for predicting weight at 180 days was studied with intralitter correlations between the three weights. The intralitter basis was used in order to eliminate the effects of environmental conditions which may have varied from litter to litter as well as from season to season. The correlations were:

0.55 ± 0.02 between 180-day weight (W) and weaning weight (Y)
0.43 ± 0.02 between 180-day weight (W) and birth weight (Z)
0.48 ± 0.02 between weaning weight (Y) and birth weight (Z)

The multiple correlation coefficient with 180-day weight dependent on birth weight and weaning weight was 0.58, as compared with a simple correlation coefficient of 0.55 between 180-day weight and weaning weight. Birth weight added to the multiple correlation only a little information that was not already included in the weaning weight. The standard net regression coefficients (betas) were +0.45 for weaning weight and +0.21 for birth weight or, in terms of a score card, weaning weight is twice as useful as birth weight in predicting weight at 180 days. The part of variance in 180-day weight that would have disappeared if both birth weight and weaning weight had been held constant was 33.6 percent. Weaning weight accounted for 20.2 of this, birth weight 4.3, and the joint effects of birth weight and weaning weight 9.1.

STATISTICAL STUDY OF HERITABILITY

REGRESSION OF THE VARIANCE IN 180-DAY WEIGHTS ON THE GENETIC RELATIONSHIP BETWEEN PIGS

Related individuals are likely to have received some of the same genes from common ancestors and for that reason they may be expected to be more alike in their hereditary traits than are unrelated individuals. The variance remaining within a pair whose members are genetically identical is that due to environment. Wright's (*10*) coefficient of relationship measures the probable genetic likeness between individuals related in various ways by descent. By computing the regression of the variance between pigs on their relationship to each other in an actual population of pigs showing a considerable range of relationships to each other, it is possible to estimate how much variance among unrelated pigs in this population was caused by differences in their heredity and how much by differences in their environment.

TABLE 3.—*Illustration of the intralot pairing of pigs for computing the regression of the variance in 180-day weight on the genetic relationship between pigs*

Pigs paired	Relationship coefficient	Variance in 180-day weight
		Pounds
185 and 145	0.206	128
323 and 252	.192	288
94 and 200	.451	32
121 and 175	.096	180
82 and (121+175)	.206	140
(94+200) and (323+252)	.266	1,936

In arranging the data for this part of the analysis, only pigs that were kept in the same lot were compared. This was done in order to eliminate as much as possible of the variation due to controllable environment. No pig was compared with a litter mate. This restriction on the pairing was made to insure that differences between dams would exist in every comparison. Thus the larger common environment of litter mates than of nonlitter mates was not permitted to affect the regression. One of the pairs in each lot consisted of the two most closely related pigs and another consisted of the two least closely related pigs. The remaining individual pigs were paired at random. If odd pigs left over from pairing were present, they were matched with one of the pairs, then one pair was matched with another pair. Groups of three or more could have been paired with each other to use all the

information about the intralot variance in 180-day weight; however, the extra information gained by including these complex comparisons was not thought to be worth the extra computation necessary. Table 3 illustrates this pairing in one lot of nine pigs. In the first four lines the relationship coefficient is simply Wright's (*10*) coefficient of relationship between two individuals related by descent. Pigs 121 and 175 were paired because they were the least closely related pair in this lot, while pigs 94 and 200 were the most closely related. In the fifth line the relationship is the probable correlation between the genotype of pig 82 and an average of the genotypes of pigs 121 and 175, while the variance similarly is that corresponding to the one degree of freedom between the weight of pig 82 and the average weight of pigs 121 and 175.

Figure 3 is a path-coefficient diagram showing how the correlation

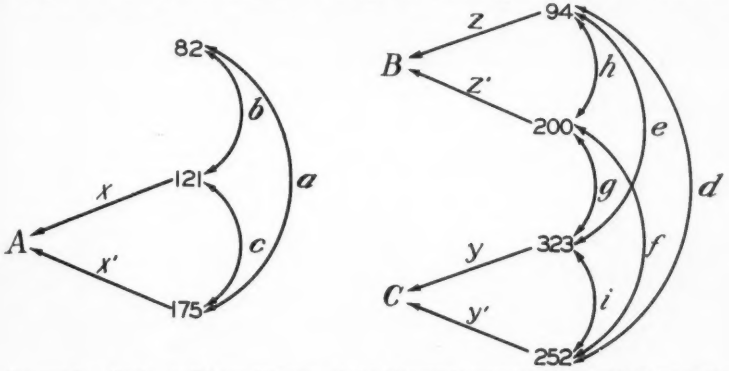


FIGURE 3.—Path-coefficient diagram of the biometric relations involved in the genotypic correlation between one pig and the average of two others, and between the averages of two pairs of pigs: Correlation (left) of pig 82 with A, the average of pigs 121 and 175; correlation (right) of B, the average of pigs 94 and 200, with C, the average of pigs 323 and 252.

between the genotype of pig 82 and A was completed. $r_{82\times A}= bx+ ax'$ which$=\frac{a+b}{\sqrt{2(1+c)}}$ if $x=x'$. Now $x=\frac{\sigma_{121}}{\sigma_A}$ and $x'=\frac{\sigma_{175}}{\sigma_A}$. These need not be exactly identical but will not vary much since $\sigma_{121}=\sqrt{1+F_{121}}$ times the genetic standard deviation in the population at the base date to which pedigrees were traced for computing relationships, and $\sigma_{175}=\sqrt{1+F_{175}}$ times the same standard deviation. The range in inbreeding coefficients was not enough to cause x and x' to be very different in most pairs in this population. For convenience $r_{82\times A}$ was computed by the above formula which required only the substitution of $a=0.113$, $b=0.193$, and $c=0.096$ to yield 0.206 in this case.

In a similar way the correlation between genotypes in a comparison of two pigs with two others (fig. 3, right) is as follows:

B=the average genotype of pigs 94 and 200, and C is the average genotype of pigs 323 and 252.

$r_{BC}=zey+zdy'+z'gy+z'fy'=$

$$\frac{\text{the sum of four covariances of 94 and 200 with 323 and 252}}{\sigma_B\sigma_C}$$

It seems approximately correct to assume that $z=z'$ and $y=y'$ but not that $z=y$, since i and h may be quite different. Some pairs were selected for the very reason that they were slightly correlated genotypically and others were chosen because they were highly correlated. Therefore r_{BC} was computed as equal to $\frac{d+e+f+g}{2\sqrt{(1+h)(1+i)}}$ and in figure 3 (right), where $d=0.128$, $e=0.132$, $f=0.256$, $g=0.183$, $h=0.451$, and $i=0.192$, $r_{BC}=0.266$, which is shown in the bottom line of table 3. The figures in the last column of table 3 are mean squares corresponding to each individual degree of freedom, and were obtained by dividing the squared difference in a (1—1) comparison by 2, in a (twice 1—2) comparison by 6, and in a (2—2) comparison by 4 (*7, pp. 291–295*).

Table 4 shows an analysis of covariance for the 842 independent intralot comparisons. The average regression equation, based on the pooled intralot variances and convariances and the general means, was $V=801.7-240.5R$. This straight line is plotted in figure 4.

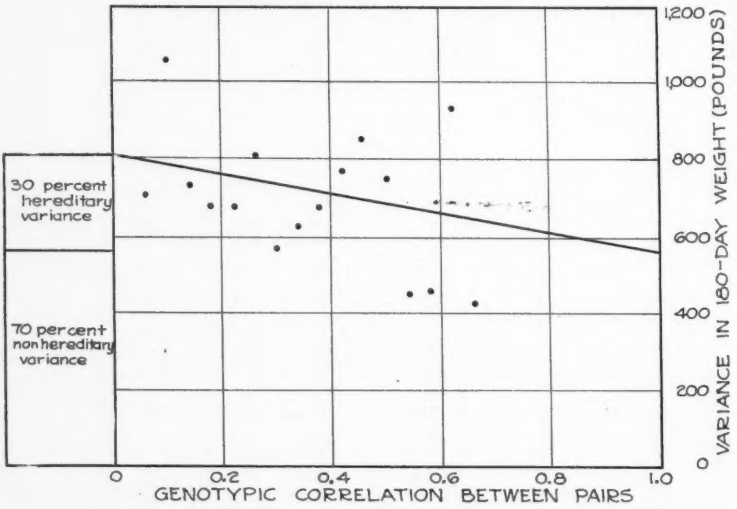


FIGURE 4.—Regression of the variance in 180-day weights on the relationship between members of 842 pairs of pigs.

The standard error of this regression coefficient (—240.5) is approximately 33. For each 1 percent increase in relationship there was an average decrease of 2.4 in the variance between pairs.

From this regression equation, $V=801.7$ when $R=0$. This would be the variance expected between unrelated pigs which were lot mates and would include all the variance due to their differences in heredity plus all the variance due to environmental variations, known and unknown, within the lots in these experiments. Substituting $R=1.0$, yields $V=561.2$ as the variance expected within an isogenic line kept under the same conditions as these pigs were kept. Hence, $\frac{801.7-561.2}{801.7}=0.30$=the fraction of the original variance which was due to differences in heredity.

Because most of the relationships on which this regression line was fitted were low, and because the line curves downward as R increases if dominance exists and if there are epistatic combination effects among the genes affecting 180-day weights, this method overestimates the variance which would remain in an isogenic line and hence underestimates heritability. No basis for estimating the magnitude of this discrepancy is apparent. All that can be said concerning it is that this estimate of 30 percent includes all of the additively genetic part of the variance plus an uncertain but probably small fraction of that due to dominance and epistasis.

TABLE 4.—*Regression of variance in 180-day weight* (V) *on genetic relationship* (R) *among lot mates, maternal sibs excluded*

Source of variance	Degrees of freedom	Sum of squares for relationship (R)[1]	Sum of cross products (RV)	Sum of squares for variance (V)[2]	Regression coefficient
Between lots	86	1.91	+1,382	209,231,269	
Within lots	756	10.80	−2,598	634,651,075	−240.5
Total	842	12.71	−1,216	843,882,344	−95.6

[1] Mean R=0.340.
[2] Mean V=719.9.

Each relationship between genotypes is a statement of the degree of likeness which would most probably result from the normal operation of the Mendelian laws of inheritance and is not an actually measured observation. For example, the computed relationship between pigs 145 and 185 was 0.206 but if the actual identity of all their genes could be known, each weighted by how much it affects 180-day weight, it could readily happen that 0.18 would be the real measure of likeness, while in another pair with the same coefficient the real likeness might be 0.23. This introduces some random error into the independent variable and thus tends to blur or flatten the regression and to make the estimate of heritability too low. This would be important if only a few genes were involved, as then the Mendelian sampling error would be large relative to the range. But it would be unimportant if, as seems more probable, a very large number of genes affect 180-day weight which, after all, is a complex result of a large number of body parts and physiological processes many of which in turn are themselves genetically complex.

It should also be remembered that the environmental variance here is that within lots. In a population from many lots and many years there would almost certainly be a larger environmental variance and correspondingly lower heritability due to environmental differences between lots and between years.

INTRASIRE REGRESSION OF OFFSPRING ON DAM

From the mates and offspring of 15 boars which each sired pigs out of 2 or more sows, an intrasire correlation of 0.15 between the weight of the offspring and the weight of the dam was observed. This analysis included 756 pigs from 150 matings. The dams were themselves selected partly on their own 180-day weight and were much less variable than their offspring, as figure 5 shows. The intrasire

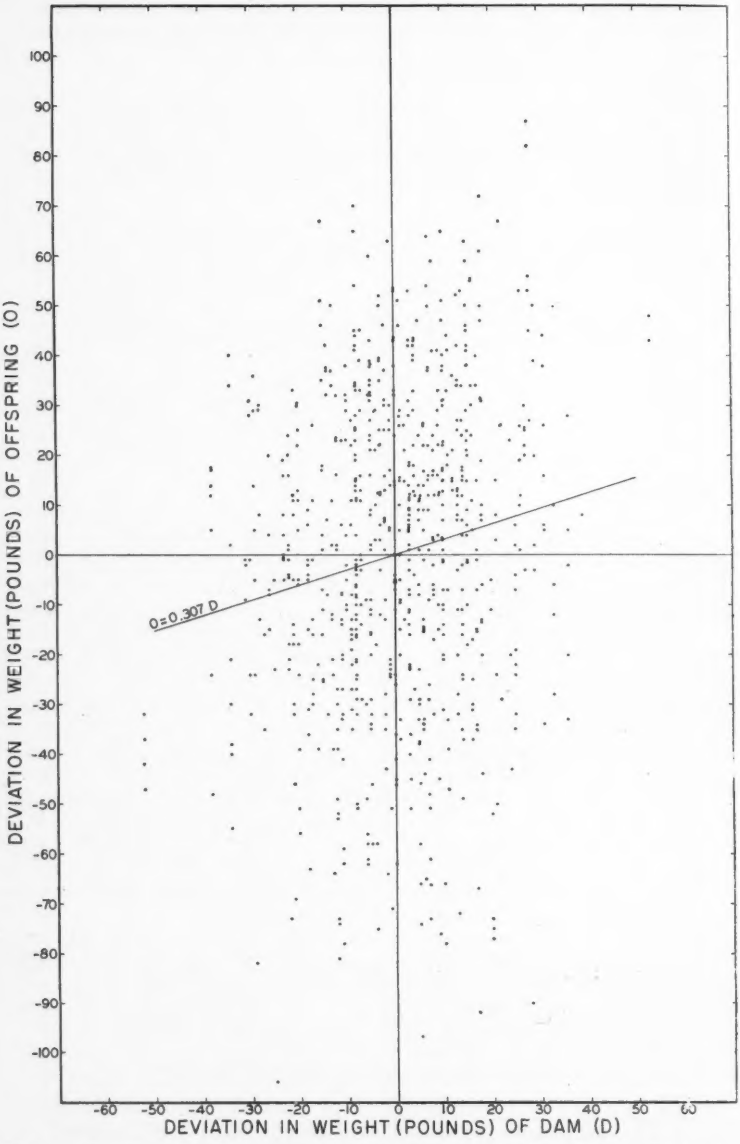


FIGURE 5.—The intrasire regression of the 180-day weight of the offspring on the 180-day weight of the dam; points plotted as deviations from the average of offspring and from the average of mates of each sire.

standard deviation of the offspring was 32.5 pounds, while that of the dams was 16.0 pounds. This smaller variation in the dams' weights makes it necessary to use regression of offspring (the unselected variable) on dam (the selected variable), rather than the correlation between them (*9*), as a basis for estimating heritability. The intrasire regression coefficient of the weight of the offspring on the weight of the dam was 0.31, which is to say that a difference of 1 pound between the 180-day weights of 2 sows mated to the same boar on the average resulted in 0.31 of a pound difference in the 180-day weight of their offspring. Doubling this (because only half of the offspring inheritance comes from the dam) yields 0.62 as the heritability of differences in 180-day weights among gilts which later were mated to the same sire. This fraction includes all of the additively genetic variance plus less than half of the epistatic variance.

The regression of the offspring weight on the dam's weight is plotted in figure 5, as are the individual deviations from this line. The weight of each offspring is expressed as a deviation from the average of all offspring of that sire and the weight of the dam is expressed as a deviation from the average of all dams mated to that sire. This eliminates the differences between the means of mates and means of offspring of different sires and makes the dam-offspring pairs from the different sires visually comparable.

CORRELATIONS BETWEEN SIBS

Correlations between the 180-day weights of litter mates and of paternal half-sibs were deduced from the analysis of variance (*3*) within litters and between litters from the same sire. Differences between farrowing seasons were eliminated by analyzing each of the eight farrowing seasons separately and then combining the results into an average intraseason analysis which is shown in table 5.

TABLE 5.—*Intraseason analysis of variance of 180-day weight due to litter and sire*

Source of variance	Degrees of freedom	Sum of squares	Mean square
Between sires	29	116,991	4,034.2
Between litters by the same sire	230	495,284	2,153.4
Between litters	259	612,275	2,364.0
Within litters	1,127	740,344	656.9
Total	1,386	1,352,619	975.9

The variance in table 5 was separated as follows on the basis of sources of differences between individual pigs:

A = variance within litters (= 656.9)
B = additional variance between paternal half-sibs
C = additional variance between nonsibs

Consequently, $A+B+C$ is the variance found within random pairs of nonsibs, $A+B$ is the variance found within random pairs of paternal half-sibs, and A is the variance found between litter mates.

The mean square between litters by the same sire (2,135.4) contains A plus $\left(\frac{1,394}{267}-\frac{4.73}{1,394}\right)B$.[5] Then $B=286.8$.

[5] The figures in parentheses are the average number of pigs per litter minus the variance of litter size divided by the total number of pigs. Generally the last term is negligibly small and may be dropped without serious error. For more detailed information on this correction see Winsor and Clarke (*8*).

The mean square between sires (4,034.2) contains the mean square between litters by the same sire plus $\left(\frac{1,394}{37}-\frac{303.2}{1,394}\right)C$. Then $C=50.2$.

In percentages of $A+B+C$ the variance ascribable to each of the three sources is:

$A=656.9=66.1$ percent of total
$B=286.8=28.9$ percent of total
$C=\ 50.2=\ 5.0$ percent of total

The variance within litters was 28.9 plus 5.0 or 33.9 percent less than the total variance which would have existed in a population of pigs all from different litters and each by a different sire. This corresponds to an intraseason correlation of 0.339 for 180-day weights of litter mates.

Paternal half-sibs are alike only in that they have the same sire. The correlation between paternal half-sibs corresponds to the fraction which the C term constitutes of $A+B+C$. This was 0.051. If all the pigs had been by the same sire but out of different dams, the total variance would have been reduced 5.1 percent. Since these correlations were deduced from mean squares which differed with high statistical significance, these correlations were likewise highly significant.

The 267 litters included in this study were from 151 sows. The presence of sets of 2 or more litters from the same sow made possible the study of the correlation between maternal half-sibs. Because a sow could produce only 1 litter in 1 farrowing season, maternal half-sibs of necessity must have been born in different years or at least in different seasons of the same year; hence, it was not possible to eliminate the effect of year and season in computing the correlation between them.

TABLE 6.—*Analysis of variance of 180-day weight showing the effect of dam*

Source of variance	Degrees of freedom	Sum of squares	Mean square
Between half-sib litters from the same dam	94	228,490	2,430.8
Between full-sib litters from the same dam	22	31,385	1,426.6
Between litters from the same dam	116	259,875	2,240.3
Between dams	150	543,739	3,624.9
Between litters	266	803,614	3,021.1
Within litters	1,127	740,344	656.9
Total	1,393	1,543,958	1,108.4

In table 6 the 266 degrees of freedom between litters were broken down into 150 between dams and 116 between litters from the same dam. The mean square between litters from the same dam included some full-sib litters as well as some litters that were only half-sibs. A separate analysis was made of the 42 full-sib litters. The degrees of freedom, sum of squares, and mean squares from this analysis are included in table 6. By their use the mean square between litters from the same dam was broken down into that between half-sib litters from the same dam and between full-sib litters from the same dam.

The variance in table 6 comes from four different sources:

A = Variance within litters = 656.9 = 66.8 percent
J = Additional variance for full sibs born in different litters = 127.4 = 13.0 percent
M = Additional variance for maternal half-sibs = 66.8 = 7.0 percent
Y = Additional variance between nonsibs = 129.8 = 13.2 percent

When the dams were the same and the sires were different the variance in 180-day weight was reduced 13.2 percent (Y). This corresponds to a correlation of 0.132 between maternal half-sibs, and might be expected to consist of a genetic fraction like that between paternal half-sibs (0.051) and an environmental fraction due to permanent differences in the ability of the dams to be good mothers and nurses. Subtracting 0.051 from 0.132 yields 0.081 as an estimate of the importance of these differences between dams, which in part may have been genetic characteristics of the dams but certainly were environmental as far as each pig's own 180-day weight was concerned. $M+Y$ gives 0.202 as the correlation between full sibs not litter mates. M or 0.070 would include some dominance and some epistatic effects, along with the genetic effect of having the same sire. Using 0.051 for the latter yields only 0.019 for the increase which dominance and epistasis make in the full-sib correlation over the half-sib correlation. This indicates that they were not very important in these data, but this conclusion has a high sampling error because of the limited numbers involved, the selection practiced among parents, and because the estimate is based on differences between mean squares, each subject to a sampling error.

The correlation between litter mates is $J+M+Y=0.332$, which is almost the same as the 0.339 observed directly. Thirteen percent (J) of the total variance in 180-day weight was caused by temporary influences which affected litter mates alike but full-sib litters from the same dam differently. Much of this was probably caused by differences in age of dam, yearly and seasonal changes, incidence of infections, temporary state of health of the dam, and other external factors that affected litter mates alike. This 13 percent is clearly an environmental part of the correlation between litter mates, but some of Y may also be environmental.

In summary, the following correlations between the 180-day weights of certain sibs were observed:

Litter mates (intraseason)	0.339
Full sibs not litter mates	.202
Maternal half-sibs	.132
Paternal half-sibs (intraseason)	.051

The correlation between paternal half-sibs in a random breeding population is composed of about one-fourth of the additive genetic fraction of the variance plus a small part (probably less than one-sixteenth) of the variance due to epistatic interactions of nonallelic genes plus the environmental fraction of the variance multiplied by the correlation between the environments of paternal half-sibs (*11*). Since the paternal half-sib correlation was computed on an intraseason basis and there was no attempt to treat the progency of one sire differently from the progeny of another sire, the environmental correlation between paternal half-sibs should be close to 0. A comparison of the correlation between full sibs not litter mates (0.202) with the maternal and paternal half-sib correlations (0.132 and 0.051) indicates that the epistatic fraction of the variance in these data is small.

This, coupled with the fact that only a small part (one-sixteenth or less) of this variance is included in the paternal half-sib correlation, indicates that the epistatic part of the variance in the correlation may be disregarded without introducing much error. On the basis of these assumptions the correlation between paternal half-sibs (0.051) is multiplied by 4 to give 0.204 as the additive genetic fraction of the variance. This estimate is probably a little below the true value since the sires were to some extent themselves selected because of their own 180-day weights and this would tend to make the correlation between paternal half-sibs a little lower than would be found in a population of pigs from truly unselected parents. It should also be remembered that whatever sampling error was present in the paternal half-sib correlation was multipled by 4 in getting the figure 0.204.

DISCUSSION

The degree of heritability of a characteristic is a measure of the amount of the observed variance that can be attributed to the additive effects of genes. All methods of estimating heritability depend in some manner on the degree to which related animals resemble each other more than unrelated ones do. The different kinds of genetic relationships for estimating heritability include various amounts of the variance due to dominance deviations, epistatic interactions, or environmental similarities or differences. It is not surprising, therefore, that estimates of heritability based on different kinds of relationships should not agree exactly, because some relationships are less affected than others by environmental variations, and by epistatic or dominance effects of genes.

The different estimates of heritability of 180-day weight derived from this study are summarized in table 7. This table also includes

TABLE 7.—*Summary of hereditary variance: estimates from different sources*

Percent of hereditary variance	How derived	Remarks
(a) 20.4	4 times paternal half-sib correlation.	Very little dominance, epistatic, or environmental correlations present in this figure. Large sampling error because of the small number of sires. Selection of sires probably makes this figure a little low.
(b) 30.4	Twice the intrasire offspring-dam correlation.	Probably too low because of the reduced variability of the dams resulting from selection. Sampling error small. Includes slightly more epistatic variance than (a).
(c) 40.4	Twice the correlation between full sibs not litter mates.	Subject to large sampling error because of small amount of data. Dominance and permanent effects of dam's nursing ability would make this figure too high, but year-to-year differences would tend to make it too low.
(d) 62.0	Twice the intrasire regression of offspring on dam.	More reliable than (b) because the reduced variability of the independent variable (dam) has no effect on the regression coefficient.
(e) 30.0	Regression of variance on genetic relationship.	Includes at least a little dominance and epistatic variance, but less environmental variance than above methods since it is a fraction of differences between pigs in the same lot.

a brief description of the method of deriving each estimate and remarks on its reliability. From a study of this table it would appear that at least 30 and perhaps more than 40 percent of the individual variance in 180-day weight was due to hereditary differences in these pigs.

SUMMARY AND CONCLUSIONS

Data were analyzed to determine the influence of hereditary and certain environmental factors on the 180-day weights of 1,394 Poland China pigs in 267 litters which were out of 151 sows and by 23 boars. The average weight of the pigs was 180.3 pounds and the intraseason standard deviation was 31.3 pounds.

A correlation of —0.17 ±0. 03 between weight of pig and percent of inbreeding and a regression coefficient indicating an average decrease of 0.76 of a pound in weight for each 1 percent increase in inbreeding were observed. The multiple correlation of 180-day weight as dependent on weaning weight and birth weight was 0.58. From the multiple regression equation it was concluded that weaning weight was twice as important as birth weight in predicting 180-day weight. Seasonal differences, year-to-year changes in environment, and differences in age of dam influenced the weight at 180 days, although the effect of the dam's age was small. Gilts weighed about 4 percent less than barrows and boars at 180 days.

The intralot regression of the variance in 180-day weight on the genetic relationship between 843 pairs of pigs indicated that about 30 percent of the intralot individual variance was due to genetic factors. The intrasire regression of offspring weight on dam's weight (0.31) gave an estimate of 62 percent for the hereditary part of the variance in individual weight of gilts which later were mated to the same boar.

A correlation of 0.051 was observed between paternal half-sibs, 0.132 between maternal half-sibs, 0.152 between dam and offspring, 0.339 between litter mates, and 0.202 between full sibs not litter mates. Because the parents were rather highly selected groups, these correlations very likely were underestimates of what would occur in an entirely unselected population. The paternal half-sib correlation gave a figure of 20 percent, the correlation between full sibs not litter mates gave a figure of 40 percent, and the offspring-dam correlation gave a figure of 30 percent for the part of variance in 180-day weight due to the action of genetic factors combining additively.

The general conclusion was drawn that at least 30 percent and perhaps more than 40 percent of the individual variance in 180-day weight was due to the additive effects of genes.

LITERATURE CITED

(1) Bywaters, James H.
1937. The hereditary and environmental portions of the variance in weaning weights of Poland-China pigs. Genetics 22: [457]–468, illus.

(2) ——— and Willham, Oliver S.
1936. A method of comparing growthiness in pigs weighed at different ages and subjected to different treatments. Amer. Soc. Anim. Prod. Proc. 1935: 116–119, illus.

(3) Fisher, R. A.
1936. Statistical methods for research workers. Ed. 6, rev. and enl., 339 pp. Edinburgh and London.

(4) Lush, Jay L.
1936. Genetic aspects of the Danish system of progeny-testing swine. Iowa Agr. Expt. Sta. Res. Bul. 204, 196 pp., illus.

(5) ——— Hetzer, H. O., and Culbertson, C. C.
1934. Factors affecting birth weights of swine. Genetics 19: [329]–343, illus.

(6) SMITH, A. D. BUCHANAN, and DONALD, H. P.
1937. WEANING WEIGHT OF PIGS AND LITTER SAMPLING WITH REFERENCE TO LITTER SIZE. Jour. Agr. Sci. 27: [485]–502, illus.
(7) SNEDECOR, GEORGE W.
1937. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. 341 pp., illus. Ames, Iowa.
(8) WINSOR, C. P., and CLARKE, G. L.
1940. A STATISTICAL STUDY OF VARIATION IN THE CATCH OF PLANKTON NETS. Jour. Mar. Res. 3: 1–34.
(9) WRIGHT, SEWALL.
1921. SYSTEMS OF MATING. I. THE BIOMETRIC RELATIONS BETWEEN PARENT AND OFFSPRING. Genetics 6: [111]–123, illus.
(10) ———
1923. MENDELIAN ANALYSIS OF THE PURE BREEDS OF LIVESTOCK. I. THE MEASUREMENT OF INBREEDING AND RELATIONSHIP. Jour. Hered. 14: 339–348, illus.
(11) ———
1935. THE ANALYSIS OF VARIANCE AND THE CORRELATIONS BETWEEN RELATIVES WITH RESPECT TO DEVIATIONS FROM AN OPTIMUM. Jour. Genetics 30: [243]–256.

U. S. GOVERNMENT PRINTING OFFICE: 1942